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# CARDIAC BIOMARKERS: PAST, PRESENT AND FUTURE

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## ABSTRACT

Cardiovascular (CV) clinical trials are essential to understand the treatment effects and to follow up the natural progression of CV disease. Biomarkers play a pivotal role in understanding the disease state, risk levels, and clinical decision-making.

We review the roles that biomarkers have played in CV clinical trials and roles that CV clinical trials have played and will continue to play in the discovery and validation of biomarkers and their implementation in clinical practice. Biomarkers were once the workhorses of patient selection and endpoint definition in clinical trials; more recently, clinical trials have been the proving ground for individual biomarkers. These markers could also reflect the entire spectrum of disease from the earliest manifestations to the terminal stages. In this paper we review recent advances with the use of biomarkers and a glimpse to the history and future of cardiac markers

**Key Words:** Cardiovascular diseases, Biomarkers, Clinical trials

## INTRODUCTION

Cardiovascular disease (CVD) is a major global cause of mortality in the developed countries. Intravascular thrombogenesis, the main pathogenic mechanism of the coronary artery disease (CAD), is influenced by a complex interplay of procoagulant, anticoagulant, fibrinolytic, endothelial damage/dysfunction and inflammatory processes [1]. The traditional theory for causation of CAD centers on a complex interplay between genetic and environmental, modifiable and non-modifiable risk factors setting into motion an inflammatory cascade of monocyte migration, lipid oxidation and atheromatous plaque formation [2,3]. The term biomarker is an abbreviation for “biological-marker” a phrase first introduced in 1989. In 2001, the definition of biomarker was refined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes or pharmacologic responses to a therapeutic intervention” [4].

Myocardial infarction (MI) can be recognized by clinical features, including electrocardiographic (ECG) findings, elevated values of biochemical markers (biomarkers) of myocardial necrosis, and by imaging, or may be defined by pathology. It is a major cause of death and disability worldwide. MI may be the first manifestation of coronary artery

disease (CAD) or it may occur, repeatedly, in patients with established disease [5].

Biomarkers are measurable and quantifiable biological parameters that can have an important impact on clinical situations. Ideal biomarkers are those that are associated with disease clinical endpoints in observational studies and clinical trials, and in some cases, they may even be used as surrogate endpoints. Biomarkers must also be both independent of established risk factors and recognized to be a factor in the disease for which they are a marker [6, 7].

Cardiac biomarkers are protein components of cell structures that are released into circulation when myocardial injury occurs. They play a pivotal role in the diagnosis, risk stratification, and treatment of patients with chest pain and suspected acute coronary syndrome (ACS) as well as those with acute exacerbations of heart failure [8, 9].

## Overview of Clinically Relevant Cardiac Markers

Clinical perspectives on biochemical markers for myocardial necrosis evolved during the 1980s and 1990s. In the past, measurement of nonspecific markers such as aspartate transaminase, lactate dehydrogenase (LD), and total Cre-

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atine kinase (CK) were performed. Subsequently, measurement of LD isoenzymes and more cardiac-specific enzymes (CK-MB) became available. LD isoenzymes and CK-MB traditionally were measured by labor-intensive electrophoretic techniques. During the 1990s, electrophoretic methods were replaced by CK-MB mass assays using automated immunodiagnostic instruments that could perform testing faster, more frequently, and at lower cost than older methods. Mass assays for CK-MB became the standard of care for cardiac marker testing in the mid-1990s. Subsequently, newer markers of myocardial injury, including CKMB isoforms, myoglobin, and cTnT and cTnI have become available on automated commercial instruments.

For the past 40 years, the use of biomarkers has been extremely valuable in the early diagnosis of acute myocardial infarctions (AMI). Sensitivity, specificity and the clinical utility have continued to increase and current research suggests that this trend will continue. This article will review the use of previous and current AMI markers and will conclude with a review of promising new markers.

### AMI Biomarker protocol

To detect MI markers, venous blood is routinely drawn from patients with chest pain who are suspected of having symptoms of acute coronary syndrome (ACS). The marker of interest is presumed to be released from the cardiac tissue which is under ischemic stress and thus may be detected in the blood sample. A detected elevation in a particular marker may lead to early diagnosis and treatment and thus improved patient outcome [10].

Characteristics of biomarkers center around three main elements, namely, kinetics of release, sensitivity and specificity. An ideal marker of cardiac necrosis should exhibit the following characteristics: cardiac specificity, early and stable release after necrosis, predictable clearance and be measurable quantitatively using rapid, cost effective methodologies available in clinical laboratories [11, 12]

The biomarkers that were proved to predict heart failure could be divided into six categories according to their origin or effects (inflammation, oxidative stress, extracellular matrix remodeling, neurohormones, myocyte injury, myocyte stress), and a seventh category of new biomarkers that have not yet been fully characterized [13].

The diagnosis of AMI can be established on the basis of these assays as early as 1.5 to 3 hours after the onset of symptoms. Several biochemical markers for the early detection of myocardial damage have been proposed, of which cardiac troponin T (cTnT), myoglobin and creatine kinase-MB (CK-MB) are the most promising candidates. Serum levels of these markers change rapidly in the early hours after the onset of AMI, therefore, sensitivity and specificity of

any particular marker change rapidly over time. Infarct size may influence early sensitivity and specificity of the cardiac marker under study [14].

Certain enzymes (CPK, LDH, etc.) are released from the heart muscle cells when it is injured ("heart attack"). These enzymes are normally found in the blood at low levels. The abnormal elevation of these enzymes in the blood stream can occasionally be the only indicator that a heart attack (myocardial infarction) has occurred.

### Biomarkers Past

Over the years, blood – based biomarkers have played an increasing number of important roles in clinical trials. In addition to refining our understanding of CVD mechanisms, they assist in both identifying study populations and defining intermediate end points (infarct size, suppression of inflammation) in phase 2 clinical trials and in identifying nonfatal clinical end points (e.g., MI) in later-phase work. The earliest blood biomarkers of cardiac injury and disease were activity-based assays to cytosolic myocardial enzymes. These included aspartate aminotransferase (AST) which was the first, lactate dehydrogenase (LDH), and creatine kinase (CK). Beginning in the 1950s, these tests were added to the expanding collection of rapid, automated clinical chemistry assays. These enzymatic assays were found to be of most use as screening tests for ischemic myocardial necrosis brought about by acute myocardial infarction (AMI). These first-generation tests lacked cardiac tissue specificity, being present also in skeletal muscle and other tissues. They furthermore lacked sensitivity, with relatively high baseline values that made interpretation of small increases in serum enzyme activity difficult. These older enzymatic assays performed even worse when used to screen for non-ischemic diseases. An important advance in cardiac biomarkers was the invention and use of monoclonal antibody strategies in the 1980s and subsequent automated immunoassay techniques. Coming from this improvement was the antibody based CK-MB assay and other tests. This began a new generation of cardiac biomarkers that were antibody-based, and resulted in an immediate improvement in the diagnosis of AMI. Again, however, these early immunoassays were still of inadequate sensitivity to be of value for more chronic heart diseases that do not cause substantial myocardial necrosis, such as valvular insufficiency, septal wall defects, and cardiomyopathies [15].

### Inflammation

Inflammation is important in the pathogenesis and progression of many forms of heart failure, and biomarkers of inflammation have become the subject of intense inquiry. All stages of plaque development and eventual rupture leading to acute coronary syndromes can be considered an inflam-

matory response. The detection of key molecules involved in the atherosclerotic inflammatory cascade therefore offers an attractive approach for detecting cardiac ischemia and predicting outcomes [16].

### C-reactive protein

Interest in the presence of inflammatory mediators in patients with heart failure began in 1954, when a crude assay for C-reactive protein, a protein that appears in the serum in a variety of inflammatory conditions, became available. A study published in 1956 reported that C-reactive protein was detectable in 30 of 40 patients with chronic heart failure and that heart failure was more severe in those with higher levels of C-reactive protein [17]. Subsequently, C-reactive protein was described as an acute-phase reactant synthesized by hepatocytes in response to the proinflammatory cytokine interleukin-6. The use of C-reactive protein as a biomarker became more common when a low-cost, high-sensitivity test for C-reactive protein was developed [18]. Multivariate analysis indicated that increased C-reactive protein level is an independent predictor of adverse outcomes in patients with acute or chronic heart failure. In the Framingham Heart Study, for example, C-reactive protein (as well as the inflammatory cytokines interleukin-6 and tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ]) was noted to identify asymptomatic older subjects in the community who were at high risk of the future development of heart failure [19]. Further, C-reactive protein has been shown to exert direct adverse effects on the vascular endothelium by reducing nitric-oxide release and increasing endothelin-1 production, as well as by inducing expression of endothelial adhesion molecules [20]. These findings suggest that C-reactive protein may also play a causal role in vascular disease and could therefore be a target of therapy.

### Pro inflammatory cytokines

In 1990, Levine et al. described elevated levels of circulating TNF- $\alpha$  in patient with heart failure [21]. TNF- $\alpha$  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. The cytokine hypothesis of heart failure proposes that a precipitating event — such as ischemic cardiac injury — triggers innate stress responses, including elaboration of proinflammatory cytokines, and that the expression of these cytokines is associated with deleterious effects on left ventricular function and accelerates the progression of heart failure [22]. Proinflammatory cytokines appear to cause myocyte apoptosis and necrosis; interleukin-6 induces a hypertrophic response in myocytes, whereas TNF- $\alpha$  cause left ventricular dilatation, apparently through activation of matrix metalloproteinases. Interleukin-6 and TNF- $\alpha$  levels could be used to predict the future development of heart failure in asymptomatic elderly subjects in one study, [23] though blockade of TNF- $\alpha$  has not resulted in

clinical benefit in patients with heart failure. [24]. Fas (also termed APO-1) is a member of the TNF- $\alpha$  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 [25]. The inhibition of soluble Fas in animals reduces postinfarction ventricular remodeling and improves survival [26]. Pharmacologic efforts to reduce Fas levels are still in their infancy but may represent a new direction in the treatment or prevention of heart failure. Indeed, the administration of a nonspecific immunomodulating agent — pentoxifylline [27] or intravenous immunoglobulin [28] — 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.

### Oxidative stress

Increased oxidative stress results from an imbalance between reactive oxygen species (including the superoxide anion, hydrogen peroxide, and the hydroxyl radical) and endogenous antioxidant defense mechanisms. The imbalance can exert profoundly deleterious effects on endothelial function [18] as well as on the pathogenesis and progression of heart failure [29]. Oxidative stress may damage cellular proteins and cause myocyte apoptosis and necrosis. It is associated with arrhythmias and endothelial dysfunction, with the dysfunction occurring through reduction of nitric oxide synthase activity as well as the inactivation of nitric oxide [30]. Inflammation and immune activation, activation of the renin-angiotensin-aldosterone system and the sympathetic nervous system, and increases in circulating catecholamine levels and peroxynitrite formed from the interaction of the superoxide anion and nitric oxide all may increase oxidative stress [31]. Since it is difficult to measure reactive oxygen species directly in humans, indirect markers of oxidative stress have been sought. These include plasma-oxidized low-density lipoproteins, malondialdehyde and myeloperoxidase (an index of leukocyte activation), urinary levels of biopyrrins (oxidative metabolites of bilirubin), [32] and isoprostane levels in plasma and urine [33]. The levels of plasma myeloperoxidase [34] and isoprostane excretion correlate with the severity of heart failure and are independent predictors of death from heart failure, even after adjustment for baseline variables [35]. The urinary excretion of 8-isoprostane correlates with the plasma levels of matrix metalloproteinases, which at high levels can accelerate adverse ventricular remodeling and increase the severity of heart failure [36]. There is increasing evidence that xanthine oxidase, which catalyzes the production of two oxidants, hypoxanthine and xanthine, plays a pathologic role in heart failure [37]. Uric acid production is elevated in as-

sociation with increased xanthine oxidase activity. Elevated levels of uric acid correlate with impaired hemodynamics[38] and independently predict an adverse prognosis in heart failure[39].

### Extracellular-matrix remodeling

Remodeling of the ventricles plays an important role in the progression of heart failure [40]. The extracellular matrix provides a “skeleton” for myocytes and determines their size and shape. Normally, there is a balance between matrix metalloproteinase (proteolytic enzymes that degrade fibrillar collagen) and tissue inhibitors of metalloproteinase. An imbalance, with dominance of matrix metalloproteinase over tissue inhibitors of metalloproteinase, is associated with ventricular dilatation and remodeling. An abnormal increase in collagen synthesis may also be deleterious to cardiac function because the resultant excessive fibrosis can impair ventricular function. The propeptide procollagen type I is a serum biomarker of collagen biosynthesis. Querejeta *et al* [41] observed a positive correlation between the serum level of propeptide procollagen type I and the fractional volume of fibrous tissue determined from cardiac biopsies in patients with essential hypertension. Cicoira *et al* [42] reported that the level of plasma procollagen type III in patients with heart failure is an independent predictor of adverse outcomes.

Thus, elevated markers of increased extracellular-matrix breakdown on the one hand and of excessive collagen synthesis on the other are associated with impaired left ventricular function and adverse clinical outcomes in patients with heart failure. Markers of these processes appear to be important targets of therapy. However, at least 15 matrix metalloproteinase and several forms of procollagen and of tissue inhibitors of metalloproteinase have been identified [43].

### 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 [44]. The urinary excretion of norepinephrine was also increased[45]. 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. Cohn *et al* [46] subsequently demonstrated that plasma norepinephrine level was an independent predictor of mortality. Swedberg *et al* [47] made the important observation that the renin–angiotensin–aldosterone system becomes activated in patients with heart failure as well.

Subsequently, after its discovery, attention focused on big endothelin-1, a 39-amino-acid prohormone secreted by vascular endothelial cells that is converted in the circulation into the active neurohormone endothelin-1, a peptide hormone 21 amino acids in length. Endothelin-1 is a powerful stimulant

of vascular smooth-muscle contraction and proliferation and ventricular and vessel fibrosis and is a potentiator of other neurohormones [48]. The plasma levels of both endothelin-1 and big endothelin-1 are increased in patients with heart failure and correlate directly with pulmonary artery pressure,[49] disease severity, and mortality[50]. The Valsartan Heart Failure Trial (Val-HeFT) investigators compared the prognostic values of plasma neurohormones (norepinephrine, plasma renin activity, aldosterone, endothelin-1, big endothelin-1, and brain natriuretic peptide [BNP]) among 4300 patients [51]. 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. However, trials involving several endothelin-1–receptor antagonists have failed to show any beneficial effects on clinical outcomes [48].

In the Randomized Aldactone Evaluation Study (RALES) of patients with severe heart failure, Zannad *et al* [52] found that administration of the aldosterone blocker spironolactone was associated with a reduction of plasma procollagen type III and clinical benefit, but only in patients whose baseline levels of the procollagen were above the median. Administration of spironolactone in patients with acute myocardial infarction reduced myocardial collagen synthesis, as reflected by plasma procollagen type III, as well as postinfarct adverse left ventricular remodeling [53]. Taken together, these findings suggest that limiting the synthesis of the extracellular matrix might be an important component of the beneficial action of spironolactone in patients with severe heart failure. Arginine vasopressin is a nonapeptide that is synthesized in the hypothalamus and stored in the posterior pituitary gland and that has antidiuretic and vasoconstrictor properties. Excess release of arginine vasopressin intensifies heart failure associated with dilutional hyponatremia, fluid accumulation, and systemic vasoconstriction. Whereas plasma levels of arginine vasopressin are elevated in patients with acute or chronic heart failure [54] and are associated with poor clinical outcomes, blockade of the vasopressin 2 receptor relieves acute symptoms but does not appear to alter the natural history of severe heart failure [55].

Although the various neurohormones are distinct, they have common features. Norepinephrine, angiotensin II, aldosterone, endothelin-1, and arginine vasopressin are vasoconstrictors, thereby increasing ventricular afterload. The facts that blockade of the sympathetic nervous system and of the renin–angiotensin–aldosterone system are cornerstones of current pharmacologic treatment of heart failure support the concept that several of these biomarkers are probably part of the direct causal pathway for heart failure [55].

### Myocyte injury

Myocyte injury results from severe ischemia, but it is also a consequence of stresses on the myocardium such as inflam-

mation, oxidative stress, and neurohormonal activation. During the past two decades, the myofibrillar proteins — the cardiac troponins T and I — have emerged as sensitive and specific markers of myocyte injury and have improved greatly the diagnosis, risk stratification, and care of patients with acute coronary syndromes.

Modest elevations of cardiac troponin levels are also found in patients with heart failure without ischemia[56]. Horwich *et al* [57] reported that cardiac troponin I was detectable ( $\geq 0.04$  ng per milliliter) in approximately half of 240 patients with advanced, chronic heart failure without ischemia. In this issue of the Journal, Peacock *et al* [58] report that troponin measurements are a predictor of outcome in hospitalized patients with acute decompensated heart failure. Other myocardial proteins — including myosin light chain 1, heart fatty-acid binding protein and creatine kinase MB fraction — also circulate in stable patients with severe heart failure. Like cardiac troponin T, the presence of these myocardial proteins in the serum is an accurate predictor of death or hospitalization for heart failure [59]. Future studies should compare the predictive accuracy of troponin measured with a high-sensitivity assay and the predictive accuracy of these other biomarkers of myocyte injury to determine whether the latter add information.

## Myocyte stress

### Natriuretic Peptides

The precursor of BNP and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) is a pre-prohormone BNP, a 134-amino-acid peptide that is synthesized in the myocytes and cleaved to the prohormone BNP of 108 amino acids. The prohormone is released during hemodynamic stress — that is, when the ventricles are dilated, hypertrophic, or subject to increased wall tension[60]. 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. Thus, when considered together, the actions of BNP oppose the physiological abnormalities in heart failure.

The natriuretic peptides are cleared by the kidneys, and the hypervolemia and hypertension characteristic of renal failure enhance the secretion and elevate the levels of BNP, especially the NT-pro-BNP[60]. There is also a moderate increase in the level of circulating BNP with increasing age, presumably in relation to myocardial fibrosis or renal dysfunction, which are common in the elderly. Pulmonary hypertension from a variety of causes may increase the plasma level of BNP. The level varies inversely with the body-mass index [61].

Measurement of natriuretic peptides may also be used to screen for acute or late cardiotoxic effects associated with cancer chemotherapy [62, 63]. Two studies have directly compared BNP and NT-pro-BNP[64]. Both 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 [65].

### 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 [66]. The level of circulating adrenomedullin is elevated in patients with heart failure and is higher in patients with more severe heart failure. The midregional fragment of the proadrenomedullin molecule, consisting of amino acids 45 to 92, is more stable than adrenomedullin itself and easier to measure[67]. Khan *et al* [58] compared midregional proadrenomedullin and NT-pro-BNP levels in patients after acute myocardial infarction. Both biomarkers were equally strong predictors of cardiovascular death or heart failure. Measurements of midregional proadrenomedullin provided additional prognostic value when combined with those of NT-pro-BNP[68].

### ST2

ST2, a member of the interleukin-1 receptor family, is a protein secreted by cultured monocytes subjected to mechanical strain. The ligand for this receptor appears to be interleukin-33, which — like BNP and adrenomedullin — is induced and released by stretched myocytes. Infusion of soluble ST2 appears to dampen inflammatory responses by suppressing the production of the inflammatory cytokines interleukin-6 and interleukin-12. Elevated levels of ST2 occur in patients is associated with severe heart failure [69].

### Conclusion

Growing evidence has shown that the use of biomarkers reflects different pathologic entities, such as inflammation, oxidative stress, tissue necrosis, and platelet activation. However, no available biomarker offers ideal diagnostic properties for ACS, such as early detection, high sensitivity and specificity, easy availability, and cost effectiveness. Thus, the deployment of new strategies is essential to meet diagnostic, prognostic, and therapeutic needs. With the full use of newly emerging technologies, alone and in combination, novel biomarkers or novel biomarker protein signatures discovery is necessary.

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