REVIEW ARTICLE

# Novel Biomarkers in Cardiovascular Disease: A Review

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## Abstract

**BACKGROUND:** The investigation of novel circulating serum and plasma biomarkers in patients with cardiovascular disease has been accelerating at a remarkable pace. New markers or tests are often presented too early to the medical profession, potentially leading to overuse and, thus, extra burden and costs to patients, the healthcare industry, and the economy. The challenge for clinicians and medical researchers is how to optimally apply existing and new markers/tests.

**CONTENT:** Biomarkers are biological parameters that can be objectively measured and quantified as indicators of normal biologic processes, pathogenic processes, or responses to a therapeutic intervention. Typically thought of as disease process screening, diagnosing, or monitoring tools, biomarkers may also be used to determine disease susceptibility and eligibility for specific therapies.

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.

**SUMMARY:** Active investigation has brought forward an increasingly large number of novel candidate markers but few have withstood the test of time and become integrated into contemporary clinical care because of their readily apparent diagnostic, prognostic, and/or therapeutic utility. With regard to the more novel biomarkers, careful thought is needed with regard to the appropriate target populations for discovery and validation, as well as the criteria used to sort out the contenders from the pretenders.

**KEYWORDS:** biomarker, cardiovascular disease, atherosclerosis, acute myocardial infarction, heart failure, risk stratification, diagnosis, prognosis.

## Introduction

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in the most parts of the world (1). Primary prevention and secondary prevention of CVD are public health priorities (2). Substantial data indicate that CVD is a life course disease that begins with the evolution of risk factors that in turn contribute to the development of subclinical atherosclerosis (3,4). Subclinical disease culminates in overt CVD (5,6). The onset of CVD itself portends an adverse prognosis with greater risk of recurrent events, morbidity, and mortality (7,8).

Clinicians have used additional tools to aid clinical assessment and to enhance their ability to identify the "vulnerable" patient at risk for CVD, as suggested by a recent National Institutes of Health (NIH) panel (9,10).

Biomarkers can indicate a variety of health or disease characteristics, including the level or type of exposure to an environmental factor, genetic susceptibility, genetic

responses to exposures, markers of subclinical or clinical disease, or indicators of response to therapy. Thus, a simplistic way to think of biomarkers is as indicators of disease trait (risk factor or risk marker), disease state (preclinical or clinical), or disease rate (progression) (11). Accordingly, biomarkers can be classified as antecedent biomarkers (identifying the risk of developing an illness), screening biomarkers (screening for subclinical disease), diagnostic biomarkers (recognizing overt disease), staging biomarkers (categorizing disease severity), or prognostic biomarkers (predicting future disease course, including recurrence and response to therapy, and monitoring efficacy of therapy) (12).

Steady progress in the discovery of new biomarkers and evolution toward more sophisticated clinical applications offer promising possibilities to enhance the care of patients. At the same time, the remarkable increase in the pace of reports about new biomarkers demands even greater attention to their rigorous individual and comparative assessment (13).

Reports of novel biomarkers are abundant in the medical literature. These markers range from simple blood or urine markers to those obtained from, e.g., genomics, proteomics, and imaging techniques; and they vary in accuracy, invasiveness of measurement, and cost. The challenge for clinicians and medical researchers is to optimally exploit existing and new markers or tests.

## Biomarker, Discovery and Validation

Cardiovascular biomarker research efforts have resulted in the identification of new risk factors and novel drug targets, as well as the establishment of treatment guidelines. Government agencies, academic research institutions, diagnostic industries, and pharmaceutical companies all recognize the importance of biomarkers in advancing therapies to improve public health (15).

NIH Definition Working Group established the following working definitions: (1) biomarker—a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention; (2) clinical end point—a characteristic or variable that reflects how a patient feels, functions, or survives; and (3) surrogate end point—a biomarker intended to substitute for a clinical end point (16).

The development of CVD biomarkers is challenging for several reasons. As summarized in a recent consensus document (9,10), the patient vulnerable to CVD is likely harboring a triad of abnormalities: vulnerable plaque, vulnerable blood, and vulnerable myocardium. In terms of developing biomarkers, 2 of these 3 components (vulnerable plaque and myocardium) are less directly accessible relative to the third (vulnerable blood).

The aforementioned caveats notwithstanding, 3 parallel developments have revolutionized the field of biomarker discovery. First, the completion of the Human Genome Project (17) and the HapMap Project (18) and the development of microarrays, proteomics, and nanotechnology together provide new avenues for developing exceptionally informative biomarkers of CVD, including high-throughput, highly sensitive, functional assays. Second, the advances in bioinformatics coupled with cross-disciplinary collaborations (eg, of biologists, clinicians, chemists, computer scientists, physicists) have greatly enhanced our ability to retrieve, characterize, and analyze large amounts of data generated by the technological advances noted above. Third, there is increased recognition that diseases arise out of the dynamic dysregulation of several gene regulatory networks, proteins, and metabolic alterations, reflecting complex perturbations (genetic and environmental) of the "system" (19,20).

The development of biomarkers in CVD can be thought of as consisting of 2 potential approaches: the first strategy is "knowledge-based" (deductive method), and the second one is more "unbiased" (inductive strategy). These 2 approaches are complementary rather than mutually exclusive. The knowledge-based strategy relies on a direct understanding of the biological processes that underlie the process of atherosclerosis and the evolution of its sequelae. It may consist of improving existing biomarkers to enhance their performance, or it may comprise designing assays for attractive new candidate markers informed by the biology of the disease process. The unbiased approach involves trolling through tens of thousands of molecules with the use of current technological advances to characterize the biomolecular profile of a stage of the disease (21).

The systems biology tools applied to biomarker discovery investigate the hierarchical organizational of biological information: the gene itself, the mRNA that it produces, the protein coded by the mRNA, biomodules or networks, cells, organs, individuals, populations, and ecologies (19).

Genetic biomarkers are variants in the DNA code that alone or in combination are associated with disease

susceptibility, disease expression, and disease outcome, including therapeutic responses. Single nucleotide polymorphisms (SNPs; DNA sequence variation when a single nucleotide in the genome sequence is altered) have been evaluated extensively in relation to CVD. The 2 classic complementary approaches used for relating genetic sequence variation to CVD risk are the linkage approach and the association strategy (22).

The availability of rapid, high-throughput analytical platforms has facilitated molecular phenotyping of disease states by analyzing the transcriptome. The global analysis of gene expression represents a paradigm shift from the traditional single-molecule approach to the evaluation of gene regulatory networks (23-25).

Proteomic approaches to the identification of disease biomarkers rely principally on the comparative analysis of protein expression in normal and disease tissues to identify aberrantly expressed proteins that may represent new biomarkers, analysis of secreted proteins (in cell lines and primary cultures), and direct serum protein profiling (21).

Returning from the bench to the bedside, adoption of a novel biomarker into clinical practice will likely hinge on demonstration that measuring the biomarker's concentration should trigger a clinical intervention, pharmacologic or otherwise. For prognostic biomarkers, patients at higher risk for adverse events will, by definition, be afforded greater absolute risk reduction for a given relative risk reduction from a therapy, and hence a smaller number of these patients will need to be treated to prevent an adverse event. But ideally, pathobiologically relevant prognostic biomarkers would identify a formal treatment interaction with a larger relative risk reduction and thus afford even greater absolute risk reduction.

Novel biomarkers that are true risk factors and faithfully serve as surrogates for clinical outcomes have the potential to markedly diminish the cost of drug development. For example, the ability to detect atherosclerotic plaque destabilization biochemically would allow researchers to screen and identify more easily promising new pharmacotherapeutics that could then be tested in larger and more expensive phase III trials (26).

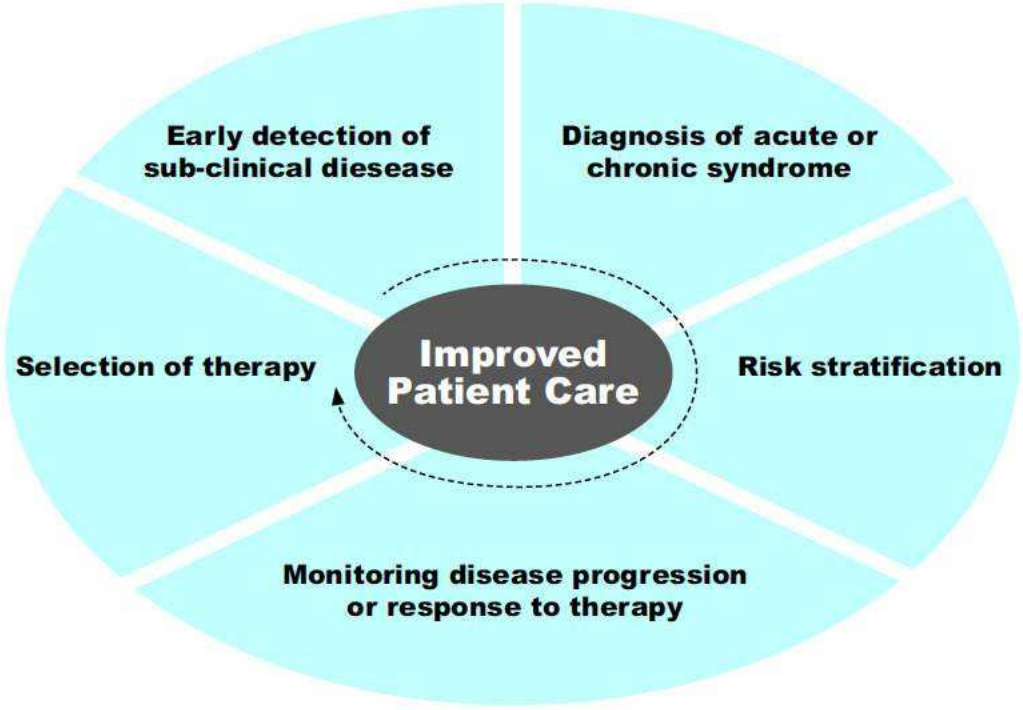


**Figure 1.** Clinical applications of cardiovascular biomarkers (Adapted with permission from Marrow DA, *et al*. American Heart Association 2007).

The clinical translation of biomarkers in the assessment of coronary atherosclerosis is affected by the complex nature of the disease with multiple cellular and humoral factors, as well as often clinically silent progression, until reaching critical ischemic or vulnerable burden, leading to myocardial damage (27).

After the discovery and verification of the candidate proteins, robust immunoassays must be developed and optimized to evaluate their potential clinical utility. Individual sandwich-based immunoassays using either monoclonal or polyclonal antibodies and nonisotopic labeled antibodies (e.g., alkaline phosphatase, fluorescein, and ruthenium) are used in this process. Although multiplexing technology is designed to simultaneously evaluate several putative biomarkers, at present the optimization of multiple protein assays is seldom achieved.

The analytical evaluation comprises several measures including trueness, accuracy, repeatability, and reproducibility, as well as a determination of linearity and limits of detection and quantification. Specific protocols for the assessment of these parameters are available to ensure a proper analytical performance evaluation of the new method (29-32). Furthermore, the frequency distribution of the candidate protein must be examined in healthy individuals to establish reference intervals to which patient results will be compared (33,34). Evaluation of the candidate biomarker in control populations will determine whether the distribution of the protein is gaussian or skewed, and whether significant differences in values exist among different age, sex, or racial subgroups. Such information is essential in determining how the reference intervals will be established.

Once the clinical usefulness of a novel marker has been demonstrated, in vitro diagnostics (IVD) companies will decide whether to pursue it commercially. Technical, medical, financial, and legal considerations will also influence this decision (35). Markers used to predict future patient outcomes, which should not be confused with markers used as surrogate outcomes. The latter use of markers requires different criteria, as recently outlined (36,37). Any marker used as a surrogate marker must have a clear and unambiguous association with subsequent patient outcomes in terms of biological or pathological processes or response to treatment.

Briefly, the process of biomarker development begins with the identification of target biomarkers with the use of standardized technology platforms, followed by validation of the assays (39,40), statistical evaluation of biomarker distributions in reference samples and in those with disease, and assessment of the correlation between biomarker levels (or expression patterns of biomarkers) and clinical measurements that define disease status (41).

## Novel Biomarkers for Cardiovascular Risk Assesement

CVD accounts for nearly 50% of all deaths in developed countries. By 2020, heart disease and stroke are projected to become the leading cause of death and disability worldwide, with the number of fatalities set to increase to more than 20 million a year. By 2030, this figure is set to increase to 23.6 million a year (41).

Currently, the only way to determine the risk for atherosclerotic pathological events is to perform annual estimates of the overall risk of CVD over 10 years. This is accomplished using traditional risk factors, such as age, sex, cholesterol level, diabetes or hypertension, in predetermined scoring models, such as the Framingham's model or the European Systematic Coronary Risk Evaluation (SCORE) model. Typically, this screening is routine from the age of 40 years in men and 50 years in women.

However, up to 50% of patients with CVD do not have high cholesterol or other traditional risk factors and, thus, are not recognized as being at risk (42). This means that approximately 30% of heart attacks are never predicted. Among these, many patients had previously been considered as being at medium risk, so were never prescribed treatment.

The ability to better predict the risk of cardiovascular events is one of the most important steps in reducing CVD and mortality. Most crucial to this is the ability to assess the risk of healthy individuals and detect atherosclerosis early – the largest target population for new biomarkers. Therefore, there is a strong need in the cardiovascular field for novel diagnostic tools that could help physicians to determine who is the most crucial to treat and to adapt their treatment strategy accordingly. Atherosclerosis is a chronic, complex and multifactorial disease. The consensus view among clinicians tends to be that a multimarker test could bring better detection of the vulnerable plaque than a single marker alone (43).

The multinational MONICA, Risk, Genetics, Archiving, and Monograph (MORGAM) (http://www.ktl.fi/morgam) project was initially established to develop cardiovascular risk scores based on classic risk factors and to determine whether genetic variability or biomarker assessment could improve on them. An overall summary of the project's objectives and detailed descriptions of the cohorts have been published elsewhere (15,16).

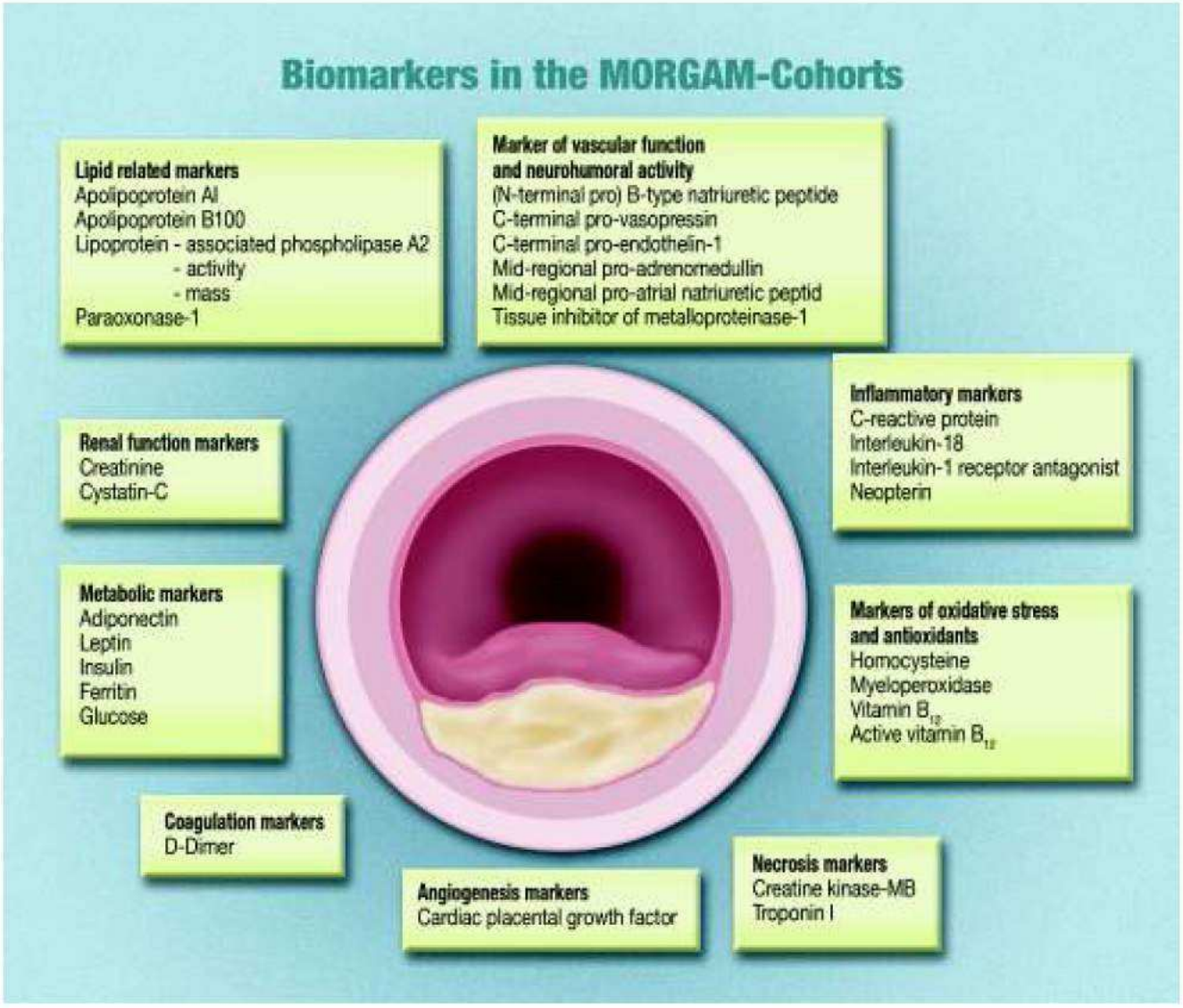


**Figure 2**. Biomarkers determined in the MORGAM cohorts (Adapted with permission from Blakenberg S, *et al*. American Heart Association 2010).

MORGAM Biomarker Project conclude that the addition of NT-proBNP, C-reactive protein, and troponin I to an established risk model improves 10-year risk estimation for nonfatal and fatal cardiovascular events in middle-aged European populations (46).

Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) are well-established independent risk factors for cardiovascular disease (47). Several clinical trials have shown that lipid-lowering drugs aimed at LDL cholesterol (LDL-C) 8 reduce cardiovascular events by 30%–45% (48,49). The large residual risk in treated individuals may be partially explained by low HDL-C (50), but recent reports have suggested that increased HDL-C does not always protect against cardiovascular disease (51) and can sometimes be associated with increased coronary events (52).

Although epidemiologic studies have shown that low HDL-C is a negative risk factor, raising HDL-C pharmacologically has not been definitively established to protect against ischemic heart disease (IHD) (51,52). This was especially evident from the recent study of the cholesteryl ester transfer protein (CETP)-inhibitor torcetrapib, which increased HDL-C concentrations but did not reduce cardiovascular events (52). A possible explanation for these contradictory findings may be that HDL becomes "dysfunctional" and may lose some of its antiatherogenic properties (53-55).

IHD was associated with high pre-β1 HDL concentrations and low LCAT levels, yielding correct classification in more than 90% of the IHD cases for which both were measured, thus making pre-β1 HDL concentration and LCAT activity level potentially useful

diagnostic markers for cardiovascular disease (56).

Given the complexity of HDL metabolism and composition, it is perhaps not surprising that no single feature of HDL is sufficient to fully capture all of its antiatherogenic properties. We hope that in the future, tests for HDL biomarkers such as pre-β1 HDL and LCAT will increase our ability to predict IHD risk and lead to the development of new and better drugs that modulate HDL-C concentrations (56).

A central tenet of atherosclerosis has been the concept that LDL undergoes modification into oxidized LDL (oxLDL) in tissues like the arterial wall. Uptake of oxLDL by macrophages and other vascular cells incites a cascade of events that promote inflammation, atherosclerosis, and eventually plaque rupture. A major advance in this area came with the identification of the lectin-like oxidized LDL receptor 1 (LOX-1) that, upon activation by ox-LDL binding, induces multiple proatherosclerotic responses in endothelial cells (ECs) as well as smooth muscle cells and macrophages (57).

Given the connections between LOX-1 pathways and atherosclerosis, Inoue *et al.* (58) reasoned that more focused measurements of LOX-1 and its activity might predict future cardiovascular events. LOX-1 can be released from the endothelial cell surface, generating soluble LOX-1 (sLOX-1). A plausible assumption is that sLOX-1 levels correlate with endothelial LOX-1 levels. Activation of LOX-1 in humans can be evaluated by use of the LOX index, obtained by multiplying the circulating concentration of LOX-1 ligands containing apolipoprotein B (LAB) times that of the soluble form of LOX-1 (sLOX-1) [LOX index = LAB × sLOX-1] (58). Higher LOX index values were associated with an increased risk of CHD. Low LOX index values may be protective against ischemic stroke (58).

These data also serve as a model to think about where advances in science and the need for better predictive tools might take us. No doubt additional players that either protect against or promote atherosclerosis, including that which occurs in distinct vascular beds, will continue to be identified—the vector of basic biomedical research (57).

Genetic studies have succeeded in demonstrating that Lp (a) is not just a biomarker of increased risk of IHD but a causal factor. In addition, the recent large meta —analysis of individual data from 126,634 participants from 36 prospective studies demonstrates the robustness of the association between elevated levels of lipoprotein(a) and increased risk of cardiovascular disease. Together, this warrants that lipoprotein(a) should be taken much more seriously in the future, for cardiovascular risk examination and potentially as a target for drug therapy. Therefore, it is our hope that renewed interest in lipoprotein(a) may be spurred. Despite numerous past studies there are still unknowns, such as the exact mechanism of how lipoprotein(a) causes MI, is it primarily via increased atherosclerosis or increased thrombosis or a combination of both?

The soluble Phospholipase A2 (sPLA2) constitutes a family of nine enzymes in humans that generate fatty acids and lysophospholipids, and trigger a variety of proinflammatory actions. It is now well accepted that sPLA2 plays a crucial role in the formation and destabilization of atherosclerotic plaque and contributes to lipoprotein retention, foam cell formation and inflammation in a developing lesion. Furthermore, the potential clinical benefit of sPLA2 inhibitors in the treatment of CVDs has already been explored in several Phase II trials, and these molecules will enter Phase III in the coming months. Last, but not least, sPLA2 activity recently emerged as a very promising biomarker for cardiovascular risk (43).

## Novel Biomarkers for ACS

Chest discomfort is the leading symptom in patients with coronary artery disease as well as the leading cause of presentation to the emergency department (63). The initial evaluation is based on the patient's clinical history, changes in the ECG and necrosis biomarkers. Historically, glutamate oxaloacetate transaminase was found to be elevated in the blood of patients having an acute myocardial infarction (64). Since then, other biomarkers, such as creatinin kinase, myoglobin and troponin, have facilitated early and accurate detection of myocardial damage and necrosis. Among them, troponin bears the highest diagnostic accuracy and is the cornerstone of the contemporary management of acute coronary syndrome (ACS) (65). Given that troponin, as a marker of myocardial damage, detects terminal events in the cascade of ACS, there is a need to search for biomarkers that are able to identify patients at high risk, allowing rapid, bedside stratification.

### I. BIOMARKERS FOR OXIDATIVE STRESS AND INFLAMMATION IN ATHEROSCLEROSIS

Atherosclerosis has become increasingly recognized as a pathological state, characterized by the accumulation of oxidative stress and inflammation in association with lipids in the artery wall (66-68). These processes have been implicated at all stages of the disease, from the very early appearance of endothelial dysfunction, through the

propagation and rupture of atherosclerotic plaque and, ultimately, tissue injury, in the settings of both ischemia and reperfusion. Accordingly, there has been considerable interest in the monitoring of these pathways as potential biomarkers for CVD (69).

Central to the atherosclerotic process is the role of oxidized forms of low-density lipoprotein (LDL) (70,71). Following its entry and trapping within the artery wall, LDL particles are rapidly oxidized by these systems. Oxidized LDL (oxLDL) is avidly taken up by macrophages forming foam cells, the cellular hallmark of atherosclerotic plaque. Each of these pro-oxidant pathways have been demonstrated to be regulated by multiple risk factors, including hypertension, dyslipidemia and impaired glucose tolerance.

Inflammatory cells have been implicated at every stage of the disease process (68). The stage of endothelial dysfunction that precedes any pathological change within the vessel wall is characterized by upregulation and expression of a number of proinflammatory factors on the endothelial cell surface (72,72). Pathological studies have observed that the earliest atherosclerotic lesion is often a pure inflammatory lesion, consisting only of monocyte-derived macrophages and T lymphocytes (74). It is only after they are recruited into the artery wall that monocytes undergo a morphological change to become macrophages and express scavenger receptors that bind modified lipoproteins (68,71). These macrophages subsequently become lipid-laden foam cells by engulfing modified lipoproteins.

Recent work has identified specific inflammatory pathways, regulated by myeloperoxidase (MPO) and various members of the phospholipase family that mediate a range of pathological events within the artery wall (75,76). The elucidation of the factors involved in the promotion of oxidative and inflammatory pathways in the artery wall provides an important opportunity to develop new systemic biomarkers for potential clinical use.

### I.1. **Biomarkers for oxidative stress**

Oxidative stress biomarkers can be broadly classified in to two major categories: biomolecules damaged by ROS, and antioxidant enzymes and molecules. The first category includes molecules that are generated in a reaction with ROS.

In the process of atherosclerosis, clinically applicable biomarkers include 8 hydroxy-2´ deoxyguanosine (8 OHdG; a marker of oxidative DNA damage) (77), lipid peroxidation represented by isoprostanes (78), malondialdehyde (MDA) and thiobarbituric acid reactive substance (markers of lipid peroxidation), carboxymethyl-lysine and pentosidine (markers of glyco-oxidation), and nitrotyrosine (marker of nitro-oxidation) (79,80).

The second category consists of antioxidant enzymes and molecules that are associated with ROS metabolism. In the process of atherosclerosis, clinically applicable biomarkers include superoxide dismutase, glutamine peroxidase, catalase, heme oxygenase, thioredoxin and paraoxonase (80-82). Plasma total antioxidant status is also measured as a factor to show the general antioxidant status (83).

a) *Asymmetric dimethylarginine*

Excess ROS has been demonstrated to decrease the endothelial bioavailability of nitric oxide (NO) and promote endothelial dysfunction. Assymetric dimethylarginine (ADMA) is an endogenous inhibitor of NOS, which results in reduced NO synthesis (84,85). ADMA represents an important link between ROS and endothelial dysfunction via the reduced bioavailability of NO. ADMA decouples NOS from l arginine and, therefore, reduces NOS-derived NO production. There is also some evidence that ADMA may even cause NOS uncoupling, thereby switching NOS from NO to a superoxide-producing enzyme.

b) *Oxidized LDL (ox-LDL)*

Low-density lipoprotein is an important target of oxidation. oxLDL reflects a variety of modifications of the lipid and protein components of LDL that occur when oxygen free radicals react with polyunsaturated fatty acids and induce lipid peroxidation. oxLDL is considered to be a key step in the pathogenesis of atherosclerosis (70,71). oxLDL, with its many oxidatively modified lipids and degradation products, contributes to the pathophysiology of both the initiation and progression of atherosclerosis. oxLDL concentrations are strongly correlated with plasma LDL concentrations and, thus, the latter is a key factor in determining absolute plasma oxLDL concentration.

### I.2. **Biomarkers for inflammation**

A number of proinflammatory factors have been proven to induce the initiation and progression of atherosclerosis. Recent prospective studies have consistently demonstrated that the usefulness of these molecular proinflammatory biomarkers to predict future cardiovascular events not only in apparently healthy subjects, but also in patients with acute coronary

syndrome (86-90). Measurements of these biomarkers carry important prognostic information, independent of traditional risk factors

a) *C-reactive protein (CRP)*
CRP is predominantly synthesized in hepatocytes as an acute-phase reactant and is transcriptionally driven by IL 6, with synergistic enhancement by IL 1. It is also suggested that CRP is produced locally in vascular smooth muscle cells and macrophages in atherosclerotic lesions (68). CRP has initially been considered as a reactant of inflammation, however, recent evidence suggests that CRP itself has direct proinflammatory effects (91-93). Several mechanisms of CRP that may contribute to the initiation and progression of atherosclerosis have been reported, including promoting endothelial dysfunction, activation of circulating monocytes, induction of prothrombotic state and increased uptake of oxLDL (94).

b) *Myeloperoxidase (MPO)*
Myeloperoxidase, one of the enzymes of the innate immune system, is considered to be a bactericidal agent (76). MPO are derived from neutrophils and monocytes, and now have been identified in human plaques. A number of studies demonstrated the importance of MPO in CVD (76,96). In the system of LDL oxidation, several studies reported that MPO-generated reactive nitrogen species mediate LDL protein nitration, initiate LDL lipid peroxidation, and convert the lipoprotein into a form that promotes cholesterol deposition in macrophages and foam-cell formation (97). In addition to its role in foam-cell formation, MPO has been identified as a catalytic sink for NO (98). More recently, it has been demonstrated that MPO is a critical factor involved in the generation of impaired protective biological properties of HDL(99). Dysfunctional HDL particles lack atheroprotective properties and promote proinflammatory effects.

c) *Lipoprotein-associated phospholipase A2 (Lp-PLA2)*
Lp-PLA2 is an enzyme, a member of the phospholipase A2 superfamily and is produced by monocytes, T lymphocytes and mast cells. Lp-PLA2 is formed in the hydrolysis of oxLDL and results in a formation of lysophosphatidylcholine, a proatherogenic and inflammatory mediator (100). In plasma, approximately 80% of Lp-PLA2 is bound to LDL, and the remaining 20% is linked to HDL and very-low-density lipoprotein. On the other hand, several studies support an anti-inflammatory function of Lp-PLA2 (99,100). The protein has been demonstrated to play a role in the hydrolysis of the platelet-activating factor and manifests a possible antiatherogenic effect when high levels of Lp-PLA2 are associated with HDL in mice (101). However, at least in the clinical studies, Lp-PLA2 has demonstrated an association with the risk of CVD (102).

d) *Soluble CD40 ligand (sCD40L)*
The potent immune mediator CD40 and its counterpart CD40 ligand (CD40L) participate in numerous inflammatory pathways that contribute to multiple pathophysiological processes (103, 104). CD40/CD40L have been demonstrated to be coexpressed by all major cells implicated in atherosclerosis, including activated T lymphocytes, endothelial cells, smooth muscle cells and monocyte/macrophages. Both the receptor and the ligand are functional and CD40/CD40L interactions enhance the expression of various proatherogenic molecules, such as adhesion molecules, chemokines, cytokines, growth-factors and matrix metalloproteases. Circulating soluble CD40L has been suggested to activate endothelial cells and CD40 expressed in other cells, constitutive for the atherosclerotic plaque, and induce a proinflammatory cascade in the vessel wall.

## II. BIOMARKERS FOR PLAQUE DESTABILIZATION AND RUPTURE

Biomarkers of vulnerable plaque can potentially provide useful information for the management of patients with coronary artery disease. Ideally, they should reflect a window in the early stage of vulnerable plaque or plaque potentially prone to rupture. The identification of such a window is of immense clinical importance.

Therefore, it is reasonable to hypothesize that biomarkers reflecting reversible ischemia will identify patients with high cardiovascular risk and can be tested as surrogate markers of vulnerable plaque. Here, we described six biomarkers that have been linked to myocardial ischemia. Until now, these biomarkers of ischemia are relevant in order to exclude ischemic heart disease (high negative predictive value) but still lack specificity (105).

a) *Ischemia-modified albumin (IMA)*
IMA is one of the most thoroughly studied

**Table 1. Oxidative stress and inflammatory biomarkers in human studies reflecting cardiovascular risk (adapted with permission from Uno K *et al.* Future Science Group 2010).**

| Condition | Increase | Decrease |
|---|---|---|
| ***Atherosclerotic risk factor*** | | |
| Hypertension | 8-OHdG, oxLDL, MDA, lipid peroxides/nitric oxide | GSH, SOD |
| Diabetes Mellitus | isoprostanes, nitrotyrosine, carboxmethyl-lysine | |
| Dyslipidemia | isoprostanes, 8-OHdG | GSH |
| Metabolic Syndrome, obesity | TBARS, isoprostanes, IL-6, TNf-α, PAI-1 | adiponectin |
| ***Preclinical atherosclerosis*** | | |
| Predict future atherosclerotic disease | oxLDL, IL-6, MPO, Lp-PLA2, CRP | TAOS |
| Endothelial dysfunction | NADPH oxidase, isoprostanes, ADMA, sICAM-1 | |
| ***Clinical atherosclerosis*** | | |
| Symmetrical atherosclerosis | ADMA | |
| Coronary artery disease | oxLDL, MCP-1, MPO | |
| Acute coronary syndrome | oxLDL, sCD40L | |

8-OHdG: 8-hidroxy-2'-deoxyguanosine; ADMA: Asymmetric dimethylarginine; CIMT: Carotid intima–media thickness; CRP: C reactive protein; GSH: Reduced glutathione; Lp PLA2: Lipoprotein-associated phospholipase A2; MCP: Monocyte chemoattractant protein; MDA: Malondialdehyde; MPO: Myeloperoxidase; oxLDL: Oxidized low-denstiy lipoprotein; PAI: Plasminogen activator inhibitor; sCD40L: Soluble CD40 ligand; sICAM: Soluble ICAM; SOD: Superoxide dismutase; TAOS: Total antioxidant status; TBARS: Thiobarbituric acid reactive substance.

biomarkers approved by the US FDA for clinical use. It has been demonstrated that the capacity of albumin to bind transition metals (specifically cobalt, copper and nickel) in the setting of myocardial ischemia was diminished (106,107). During ischemia, the N terminus of albumin is modified, possibly owing to hypoxia, acidosis, free-radical injury and energy-dependent membrane disruption, therefore, decreasing its binding capacity for metals (108,109). These modifications were used to develop an albumin cobalt binding (ACB) test that measures the residual unbound cobalt fraction. This unbound cobalt fraction is used to bind a chromogen, which, in turn, can be measured photometrically (107).

The ACB test has been demonstrated to correlate with myocardial ischemia. Initial work demonstrated that changes in IMA can occur as early as 6–10 min after myocardial ischemia starts and this remains positive up to 6 h later (110,111). Thus, the ACB test detects ischemia before the development of necrosis and before an elevation of the conventional markers of necrosis (creatine kinase MB and troponin).

b) *Unbound free fatty acid (FFA)*

Total serum FFA levels provide an important measure of the physiologic state. Owing to the fact that large quantities of FFA are required to meet energy needs and since long-chain FFAs are highly insoluble in the aqueous phase, the major portion of FFA in the blood is carried in association with albumin. A small part of the total FFA within the blood, however, dissociates from the albumin. These molecules, the unbound FFA (FFAu), are in true aqueous solution as monomers of FFAu (112).

FFAu has been assessed in the setting of transient ischemia created by balloon inflation during PCI (113,114). FFAu demonstrated a rapid increase after balloon inflation. There were no significant associations between the peak FFAu level and total balloon inflation duration, ECG changes, prolonged angina, procedural complications, left ventricular function or number of lesions treated (113).

c) *Choline*

Choline is an essential component of phospholipids, plasma lipoproteins and cell membranes, and is crucial for the formation of acetylcholine. The hydrolysis of phosphatidylcholine by phospholipase D generates choline and phosphatidic acid (115). Whole-blood choline has been studied in the context

of ACS and has been demonstrated to be predictive of cardiac death during 30 days following an ACS in patients with negative troponin. In addition to whole-blood choline, serum choline has been studied in the context of ACS with troponin-negative patients, where it was shown to be predictive of cardiovascular events in conjunction with F2 isoprostane (116).

d) *Brain Natriuretic Peptide & N-Terminal proBNP (BNP & NT-proBNP)*

BNP is released upon ventricular stress together with its inert cometabolite NT proBNP (117). In the context of ischemia, it is not completely clear which stimulus launches the release of BNP. Wall stress is probably the main trigger but hypoxia itself could also be an independent trigger (118,119). Although numerous studies have demonstrated a predictive value of natriuretic peptide levels in the context of myocardial infarction (120), ACS (121) and stable coronary artery disease (122,123), few have assessed the value of BNP (NT proBNP) as a marker of ischemia. Bibbins-Domingo *et al.*, have demonstrated that elevated levels of BNP were associated with inducible ischemia and, accordingly, with an increased risk of coronary events (124).

e) *Cystatin C*

A recently published study demonstrated an association between cardiac ischemia and cystatin C (125). The association was statistically significant among patients without a history of coronary artery bypass and those who were not treated with b blockers or statins. After adjustment for baseline demographics, comorbidities, medications, creatinine clearance, cardiac function and C reactive protein, the highest cystatin C quartile predicted inducible ischemia. Although these data are interesting, the causal pathway between cystatin C and ischemia is currently an unresolved issue.

f) *High – sensitivity troponin*

Recently, a highly sensitive troponin T assay that can detect levels as low as 0.001 µg/l—ten-times lower than the conventional available test—has been developed. The mechanisms responsible for the release of very low levels of cardiac troponin T in patients with stable coronary artery disease could include transient ischemic episodes. Recently, Sabatine *et al.*, using the ultrasensitive troponin T assay, demonstrated a quantifiable rise in troponin T levels in patients undergoing stress testing, with the amount of rise being proportional to severity of

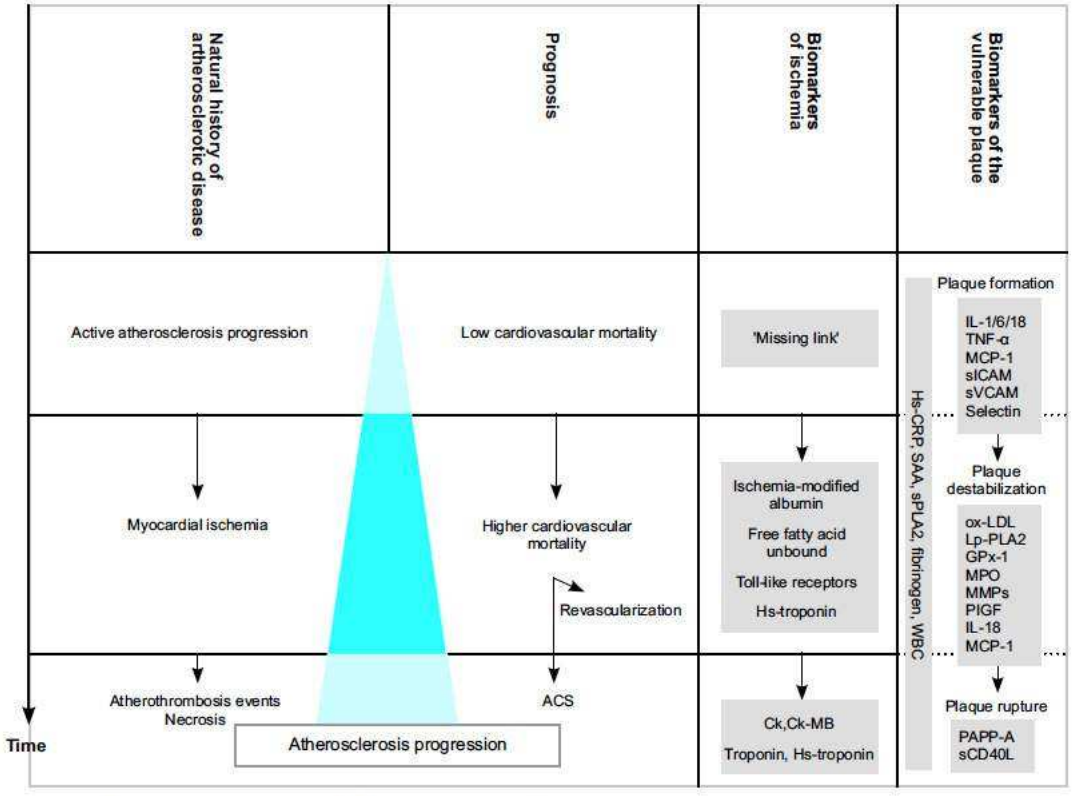


**Figure 3.** Atherosclerotic disease develops over years, beginning with fatty streaks and ending in plaque rupture (Adapted with permission from Muller O, Future Science Group 2010).

ischemia on perfusion imaging (126) Furthermore, the authors identified a rise of troponin T levels to over 0.0013 ng/ml as a strong predictor of inducible ischemia, with an odds ratio of 3.54 with 95% CI of 1.42–8.80.

## III. BIOMARKERS FOR ISCHEMIA AND NECROSIS

The diagnosis of acute myocardial infarction currently rests on the measurement of troponin, a biomarker of myocardial necrosis. Unfortunately, the current generation troponin assays detect troponin only 6–9 h after symptom onset. This can lead to a delay in diagnosis and also excessive resource utilization when triaging patients who, ultimately, have noncardiac causes of acute chest pain. For these reasons, there has been extensive research interest in biomarkers that can detect and rule out myocardial infarction early after symptom onset. These include markers of myocardial injury, such as myoglobin, heart-type fatty acid binding protein, glycogen phosphorylase BB; hemostatic markers, such as d-dimer; and finally, inflammatory markers, such as matrix metalloproteinase 9. Recently, highly sensitive troponin assays have reported an early sensitivity for myocardial infarction of greater than 95%, although at a cost of reduced specificity. The optimal strategy with which to use these novel biomarkers and highly sensitive troponins has yet to be determined, and interpretation of their results in light of thorough clinical assessment remains essential (127).

a) *Heart-type fatty acid binding protein (H-FABP)*
HFABP is a small (14–15 kDa) cytosolic protein found abundantly in cardiac tissue and to a much lesser extent in the brain, liver and intestine. Several studies have examined the role of HFABP in diagnosing MI. An early study demonstrated that the diagnostic sensitivity of HFABP was superior to that of myoglobin and creatine kinase MB, and that in patients presenting between 3 and 4.5 h after symptom onset, sensitivity could be as high as 94% (128). Further investigations found similar results in larger populations (129) and in patients with undifferentiated chest pain (130). The most recent prospective study to assess HFABP's diagnostic utility reported significantly better sensitivity of HFABP when compared with troponin T in patients presenting less than 4 h after the onset of symptoms (131).

b) *Matrix metalloproteinases 9 (MMP 9)*
MMP 9 are a family of zinc-dependent endopeptidases with the functional ability to breakdown components of the extracellular matrix (132). The role of MMP 9 in atherosclerosis and plaque rupture is supported by several investigations, such as the observations that within coronary atherectomy specimens, MMP 9 levels were 70% higher in patients with unstable versus stable symptoms (133) and that unstable plaques stain more strongly for MMP 9 than those with stable symptoms (134).

c) *Soluble CD40 ligand (sCD40L)*
The CD40 ligand is expressed on a wide variety of cells, including leukocytes and activated platelets (135,136). It is recognized to play a vital role in the inflammatory progression of atherosclerosis (137,138) as well as being part of a signaling pathway involved in the initiation of plaque rupture (139). Once activated, platelets shed a functionally enabled soluble form of the CD40 ligand (sCD40L) that enables relatively easy sampling from the peripheral blood (140). In a cohort of over 1000 patients with confirmed ACS, sCD40L was an independent predictor of 30 day death or MI, and stratified a high-risk group of patients who may benefit from glycoprotein IIB/IIIa inhibition (141).

d) *Pregnancy-associated plasma protein A* (PAPP-A)
PAPP-A in relation to ACS has been extensively investigated. PAPP-A is a member of the MMP family (142), and is frequently measured in the serum of pregnant women to help stratify risk for the presence of fetal trisomy (143). Evidence of its potential role in ACS was first reported by Bayes-Genis's group in 2001. Initial autopsy derived 'unstable plaques' compared with 'stable' plaques exhibited much stronger staining for PAPP-A. This led the group to undertake subsequent measurements of PAPP-A levels in a small number of patients with ACS, stable angina and normal controls, confirming the association between peripheral PAPP-A levels and ACS (144). Further investigation in a cohort of 644 patients attending an emergency department with chest pain, again demonstrated a significant association of elevated PAPP-A levels with ACS and, importantly, elevated levels were predictive of death or MI independent of troponin and sCD40L (145).

e) *High Sensitive Troponin Assay*
Very recently, two independent investigators have published interesting studies involving highly

sensitive troponin assays (146,147). These assays have a lower limit of detection that is below the 99th centile upper reference limit for the normal population (148-150). The sensitivity of an admission sample alone for the diagnosis of MI was as high as 95%. When comparing the AUC-ROC curve for highly sensitive and standard troponin assays, the superior discriminatory power of the highly sensitive assays was evident from the admission sample in contrast to the requirement for delayed sampling with the standard assay. Furthermore, the negative predictive value of all four assays, investigated at the 99th percentile, was between 95 and 97%, utilizing only an admission sample (147). The value of these impressive early sensitivity results may be offset by a significant reduction in specificity (151-153).

f) *YKL 40*

YKL 40 is a glycoprotein secreted *in vitro* by human cells such as activated macrophages and neutrophils in different tissues with inflammation and increased remodeling of the extracellular matrix (ECM), by arthritic or injured chondrocytes, by fibroblast-like synovial cells and by vascular smooth muscle cells (VSMCs). *In vivo* YKL 40 has been found elevated in patients with diseases characterized by inflammation, increased extracellular remodeling and ongoing fibrosis (154).

YKL 40 is named after its three N-terminal amino acids and its molecular mass of 40 kDa (155). YKL 40 is also known as human cartilage glycoprotein-39 (HC gp39) (156), breast regressing protein 39 (brp-39) (157), 38-kDa heparin-binding glycoprotein (gp38k) (158), chitinase-3-like-1 (CHI3L1) (159), chondrex (160) and 40-kDa mammary gland protein (MGP-40) (161).

YKL 40 is closely related to both the early and late phases in the development of atherosclerosis. One of the key elements in the early development of atherosclerosis is the maturation of monocytes into macrophages in the arterial intima layer. Subsequent to this the macrophages take up lipids and replicate. The lipid-rich macrophages, known as foam cells, secrete inflammatory mediators that stimulate smooth muscle cell migration and proliferation and participate in plaque development and rupture as well as thrombosis (162,163).

YKL 40 induces the maturation of monocytes to macrophages and is secreted by macrophages during late stages of differentiation and by activated macrophages (159,164-167). Macrophages in atherosclerotic plaques express YKL 40 mRNA, particularly macrophages that had infiltrated deeper in the lesion, and the highest expression of YKL 40 is found in macrophages in the early lesion of atherosclerosis (168). The last part is particularly interesting because it suggests that YKL 40 could be used as a biomarker for atherosclerosis at very early stages of the disease. Several studies of patients with AMI and/or stable CAD have demonstrated elevated levels of YKL 40 compared with healthy controls (169-174).

Two large studies have both demonstrated that YKL 40 can be used as an independent predictor for both overall and CV mortality in both individuals with or without CVD (172,174). These findings support the hypothesis, that YKL 40 is closely related to both the early and late phases in the development of atherosclerosis. It could be a measure of the inflammatory activity in coronary artery plaques and thereby an indicator of the risk of developing an acute coronary syndrome or death.

Results from these studies suggest that YKL 40 could be a new biomarker of acute and chronic inflammation in patients with stable CAD. Circulating YKL 40 may reflect the total burden of coronary atherosclerosis or identify a high-risk atherosclerosis phenotype with ongoing inflammation and atherosclerotic plaque formation.

The diagnosis of MI remains challenging. Despite the development of novel assays measuring necrosis, hemostasis and inflammation, not one single test can fully exclude or confirm the presence of ACS or MI. Recently, the combination of a marker of hemodynamic stress (copeptin) with fourth-generation troponin (175) has shown promise; however, the potential of this strategy requires further prospective validation.

## Novel Biomarkers for Heart Failure

Heart failure is associated with significant mortality, morbidity and economic cost (176). Over the past 30 years, our understanding of the pathophysiology of heart failure has advanced greatly. It is now recognized as a systemic syndrome characterized by maladaptive neurohormonal, metabolic and inflammatory processes. The recognition of these pathways has prompted the evaluation of some of their components as biomarkers in both chronic heart

failure (CHF) and acutely decompensated heart failure (177).

Established biomarkers, such as B-type natriuretic peptides, have improved the diagnosis and prognostication of heart failure patients. However, morbidity and mortality rates remain high, and the development of further biomarkers may improve the management of these patients. Novel biomarkers in heart failure should add to contemporary diagnostic and prognostic acumen and be cost effective. Much work is required to ascertain which of these candidate biomarkers or their combinations have a place in contemporary clinical practice and, if so, how they will be best utilized.

I. NEUROHORMONES

Maladaptive neurohormonal augmentation is now recognized as a major contributor to the development and progression of CHF. As our understanding of CHF pathophysiology progresses, components of novel neurohormonal pathways with putative roles, both protective and deleterious, are similarly declaring themselves as candidate biomarkers (177).

a) *Human brain natriuretic peptide (BNP)*

Human brain natriuretic peptide (BNP) is an established biomarker in heart failure. It belongs to the category of cardiac-specific markers that are released by the heart in response to myocardial stress. The peptide is synthesized as a 134 amino acid peptide (pre-proBNP) that is subsequently processed to form a 108-amino acid peptide (proBNP). This proBNP is enzymatically cleaved by corin, a transmembrane serine protease produced in cardiomyocytes to form a 76-amino acid N-terminal NT peptide (NT-proBNP) and a biologically active 32-amino acid C-terminal peptide (BNP), which are both released into the circulation. The biologically active BNP, the intact 108-amino acid proBNP and the remaining part of the prohormone NT-proBNP, all circulate in the plasma and can be measured by commercially available immunoassays. They bear diagnostic and prognostic relevance, can track the therapeutic response and testing is recommended in current guidelines (178-182).

In critically ill patients, BNP levels seem to be related to prognosis in several clinical situations, such as hypoxemic pulmonary failure (183), pulmonary embolism (184), myocardial infarction (185) or septic shock (186,187).

The natriuretic peptides are the most widely studied of the heart failure biomarkers. They form a family of peptides possessing natriuretic, diuretic and vasodilatory properties that are endogenously upregulated in heart failure. Given their greater stability in plasma, the B-type natriuretic peptides (BNPs) are now widely recognized as the current 'biomarker benchmark' in both CHF and acutely decompensated heart failure. Their established role in the diagnosis, prognosis and, maybe less convincingly, guidance of therapy in CHF, has recently been comprehensively reviewed in many journal (188).

b) *Midregional pro – atrial natriuretic peptide*

The midregional segment of the pro-atrial natriuretic peptide molecule (MR-proANP) has been shown to be more stable in the plasma than either proANP or mature ANP and is emerging as a promising biomarker in this syndrome. Several small studies have demonstrated that this peptide can reliably diagnose heart failure in acutely breathless patients with a seemingly similar performance to BNP and amino-terminal BNP (NT-proBNP) (189), and also identifies asymptomatic left ventricular (LV) systolic dysfunction in patients with coronary artery disease with a performance comparable to NT-proBNP (190).

This novel peptide provided independent prognostic information in 525 (191) and 797 (192) CHF patients, which, importantly, appears to be superior to the BNPs in both cases. Therefore, MR-proANP holds considerable promise as a diagnostic and prognostic biomarker in both acute heart failure and CHF.

c) *Midregional pro – adrenomedullin*

Adrenomedullin (ADM) is a complex 52-amino acid peptide that was initially isolated from human pheochromocytoma cells and is almost ubiquitously expressed throughout the human cardiovascular system (193). It has potent vasodilator and natriuretic properties, and both myocardial and plasma levels are raised in CHF, with plasma levels predicting mortality in CHF (194). Accurate assaying of plasma levels can be inaccurate as its half-life is short. However, an assay has been developed for the more stable midregional portion of the propeptide (195). One small observational study has suggested that plasma levels of midregional proADM are of moderate value in identifying LV systolic dysfunction in patients with coronary artery disease with a negative predictive value of 88% (196).

In a large study of 923 postmyocardial infarction patients, plasma concentrations of midregional proADM were elevated and powerfully predictive of both mortality and progression to CHF following multivariable analysis (197). Circulating levels were also increased and predictive of mortality at 24 months in 786 CHF patients, with greatest prognostic value in those with mild–moderate disease severity (198).

d) *C-terminal pro-arginine vasopressin (copeptin)*

Arginine vasopressin (AVP), or antidiuretic hormone, is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus. It is a vasoconstrictor but its prime role is in the regulation of fluid homeostasis by promoting water reabsorption in the collecting ducts of the nephron (199). Plasma AVP concentrations have long been known to be elevated in CHF (200). An assay has recently been developed for the C-terminal fragment of proAVP, also known as copeptin (201). Copeptin is raised and independently predictive of adverse LV remodeling and mortality in patients following acute myocardial infarction (202-204). It also predicted 1-year mortality in 137 patients with acutely decompensated heart failure (205). Larger observational studies in CHF patients demonstrated similar prognostic properties, which appeared to provide superior information to BNP regarding mortality across the spectrum of disease severity (206,207).

e) *Chromogranin* A *(CgA)*

CgA is a 439-amino acid member of the granin family of neuropeptides that is ubiquitously distributed throughout the CNS, endocrine and neuroendocrine systems of vertebrates and nonvertebrates (208). CgA is also synthesized in the human myocardium (209) and is the precursor for a range of N-terminal fragments with various vasoactive, metabolic and antimicrobial properties (210).

Both circulating (211) and myocardial levels (209) are raised in CHF, correlating with New York Heart Association (NYHA) class and predicting prognosis. CgA concentrations similarly predict prognosis in patients presenting with acutely decompensated heart failure (212).

f) *Apelin*

The peptide hormone apelin is also ubiquitously expressed throughout the cardiovascular system (213) and is the ligand for the G-protein-coupled receptor known as APJ (214). Studies in animals have demonstrated antihypertensive (215-217), vasodilatory (218), diuretic (219-221) and potent inotropic effects in normal and failing hearts (222-226), with apelin-knockout mice displaying significant and progressive LV systolic dysfunction (227).

In humans, plasma apelin concentrations are reduced in patients following acute myocardial infraction (228), both plasma (229-233) and myocardial (230,233,234) levels are significantly reduced in patients with advanced CHF. Small studies have demonstrated that improving or augmenting cardiac performance with LVAD (230) or cardiac resynchronization therapy (231,234) leads to a significant increase in serum apelin concentrations and myocardial apelin and APJ levels, suggesting that apelin may hold promise as a marker of reverse remodeling in CHF.

g) *Urocortin*

Urocortin I, II and III are peptides with significant structural homology to the important mediator of the pituitary-adrenal glucocorticoid axis, corticotrophin-releasing factor (CRF) (235). They are the ligands for the CRF-1 and CRF-2 receptors and exhibit inotropic (236), vasodilatory (237,238), cardioprotective (239,240) and beneficial hemodynamic effects in animal models of heart failure (241-245).

To date, the only study to investigate circulating urocortin concentrations in CHF demonstrated a significant increase compared with age—and sex—matched healthy controls (246). Further clarification of the effect of CHF on plasma urocortin concentrations is necessary to establish its potential as a biomarker in this syndrome.

## II. METABOLIC BIOMARKERS

a) *Coenzyme Q10 (CoQ10)*

CoQ10 is a quinone that is a crucial component of cellular respiration in the synthesis of adenosine triphosphate via the mitochondrial electron transfer chain (247). Myocardial CoQ10 levels have long been known to be reduced in CHF and correlate with worsening NYHA status (248). More recently, plasma CoQ10 levels were shown to be reduced and independently predictive of mortality in CHF patients (249). There is some evidence that therapeutic upregulation of CoQ10 in CHF

may result in an increase in cardiac output and LV ejection fraction (250), and the results of a large international trial are awaited (251).

b) *Adiponectin*

Adiponectin is a 244-amino acid fat-derived peptide hormone with a role in metabolic regulation. It is abundantly produced by adipose tissue and less so by the myocardium, with plasma concentrations negatively correlating with body fat and BMI (252,253). Preclinical studies have demonstrated antiapoptotic, antiatherosclerotic and antihypertrophic properties, as well as the ability to improve endothelial function (252). Plasma concentrations are reduced in patients with ischemic heart disease but are increased in patients with acutely decompensated heart failure in whom they predict prognosis (254). Adiponectin synthesis is stimulated by the natriuretic peptides (255) and, as such, myocardial production and circulating levels of adiponectin are also significantly raised in CHF patients in whom they correlate closely with BNP levels and again predict prognosis (256).

## III. NOVEL INFLAMMATORY BIOMARKERS IN HEART FAILURE

Chronic heart failure is now recognized as a chronic inflammatory state, with immune activation and proinflammatory cytokines playing instrumental roles in both the development and the progression of the syndrome (257). Recent advances have identified novel inflammatory mediators with putative roles in CHF and, therefore, a subsequent interest is emerging in the utilization of these mediators as biomarkers.

a) *ST2 & IL-33*

The receptor ST2 is a member of the Toll-like/IL-1 receptor superfamily (258) with transmembrane and extracellular isoforms, the latter of which is soluble and can circulate in the plasma (259). ST2 gene expression is significantly upregulated following acute myocardial infarction in rat cardiomyocytes (260). In 2005, the ligand for ST2 was identified as IL-33 (261). IL-33 antagonizes Ang II-induced cardiomyocyte hypertrophy and fibrosis with ST2-knockout mice, displaying greater LV hypertrophy, dilatation, fibrosis and shortened survival following aortic constriction than wild-type controls (262). Therefore, it appears that the IL-33/ST2 system plays a role in regulating the protective myocardial response to pressure overload. Serum soluble ST2 levels are raised in humans following acute myocardial infarction in whom they negatively correlate with LV ejection fraction, and independently predict both mortality and progression to CHF (260,263). Circulating ST2 levels are also a strong and independent predictor of mortality in patients with acutely decompensated heart failure (264-267), as well as advanced CHF (268). Further studies are awaited in order to determine the full utility of ST2 as a biomarker in CHF.

b) *Growth differentiation factor-15*

Growth differentiation factor-15 is a member of the TGF-β superfamily whose levels are increased by oxidative stress, myocardial ischemia and inflammatory cytokines, and protects against reperfusion injury (269). It is not normally expressed in the myocardium but is induced in animal models of pressure overload and ischemia, where it promotes antiapoptotic, antihypertrophic and antiremodeling effects (269,270). Circulating levels are significantly raised in CHF patients in whom they independently predict mortality and positively correlate with age, NYHA class and BNP, whilst negatively correlating with renal function and LV ejection fraction (271).

c) *Pentraxin-3*

This acute-phase protein is a member of the long pentraxin family and is produced by macrophages, monocytes, vascular endothelium, vascular smooth muscle and adipocytes in response to proinflammatory mediators (272). These include TNF-α, IL-1 and bacterial endotoxin. In humans, plasma pentraxin-3 levels are raised following acute myocardial infarction and were stronger predictors of 3-month mortality in 734 postmyocardial infarction patients than cardiac troponin T, creatine kinase or NT-proBNP in a multivariable analysis (273). Plasma concentrations are similarly raised in CHF and independently predict mortality in these patients (274,275).

d) *Osteopontin*

This is a glycoprotein that is expressed in cardiomyocytes and fibroblasts, and can exist as an extracellular matrix molecule or as a soluble circulating cytokine. Myocardial osteopontin levels are upregulated by Ang II (276) and are raised in both animal (277-279) and human heart failure (280,281). Circulating osteopontin concentrations

were raised in 420 CHF patients in whom they correlated with worsening NYHA class and independently predicted 4 – year mortality (282).

e) *Cardiotrophin-1*

Cardiotrophin-1 is a member of the IL-6 family of cytokines. It is expressed in various tissues including the myocardium (283) and is secreted in response to myocardial stretch (284). Preclinical studies have suggested that CT-1 is negatively inotropic (285) and antiapoptotic, and also promotes the development of LV hypertrophy (286). Plasma concentrations are raised in CHF correlating positively with LV mass, IL-6, TNF-α and negatively with LV ejection fraction 287-288). Circulating levels independently predict mortality in these patients (289).

## IV. OTHER NOVEL BIOMARKERS FOR HEART FAILURE

a) *Cystatin C*

Cystatin C is a 120-amino acid serine protease inhibitor and is ubiquitously expressed in human tissue (290). It is freely filtered at the glomerulus and, therefore, is a surrogate marker of renal perfusion and glomerular filtration. **Plasma levels are increased in patients presenting with acutely decompensated heart failure and independently predict mortality at 1 year (291)**. Plasma concentrations predict the development of CHF and all-cause mortality in patients with coronary artery disease (292). They are also independently predictive of mortality in patients with CHF and appear to be of greater prognostic value to creatinine in older patients (293,294).

b) *Troponin*

Prevalence of troponin elevation in chronic and acute decompensated heart failure is established. Prognostic significance of troponin in this clinical context is emerging. Many studies, to date, have been performed using limited multivariable analyses. Large prospective studies evaluating the incremental prognostic value of troponin over established predictors of risk are required. The combination of troponin and other predictors of risk may help to improve the challenge of risk stratification in heart failure (295)

c) *G – protein – coupled receptor kinase (GRK)*

The key role that GRKs play in GPCR signaling and modulation suggests that changes in their cellular complement and functionality would strongly affect GPCR function, as has been described in several pathological conditions, such as hypertension (HTN) (296), insulin resistance (IRES) (297), congestive heart failure (CHF) (298) and coronary artery disease (CAD) (299). The heart contains GRK2, GRK3 and GRK5, of which GRK2 is the most abundantly expressed (300,301).

At present, there is much evidence demonstrating the important role of GRK levels observed in white blood cells as a biomarker of cardiac dysfunction, in particular in CHF (302-304). Therefore, lymphocytes may provide a surrogate for monitoring cardiac GRK2 in human CHF, and measuring GRK2 in the blood of CHF patients may potentially become a marker for monitoring changes in left ventricular (LV) function and, importantly, may monitor response to therapy (305).

The increase in GRK2 levels appear to be proportionate to the decrease in cardiac function. This feature indicates the use of GRK2 as a biomarker at the time of admission in the emergency room: the higher levels of peripheral white cells content in GRK2 may help to discriminate those patients with a poor cardiac performance who are in need of more aggressive therapy (306).

d) *Resistin*

Resistin, a novel metabolic marker, has been associated with the severity of and prognosis in prevalent HF, and also helps to predict incident HF. These relationships may be mediated via insulin resistance, inflammation, direct effects on myocytes or other yet to be discovered pathways. Future roles for resistin may both help stratify risk for incidental HF, prognosticate prevalent HF, and possibly help develop newer therapies (307).

e) *Cytokines and Matrix Metalloproteinase (MMP)*

HF is accompanied by the upregulation of bioactive signaling molecules, known as cytokines, and a family of downstream proteases, MMPs. It is now apparent that these molecules contribute to adverse myocardial remodeling during HF. Elevated levels of cytokines and MMPs exist in the myocardium and can subsequently spill over into the systemic circulation (308).

Specifically, TNFRI, IL-6, MMP-2 and TIMP-1 levels were elevated in HF patients and correlated with NYHA functional status (309-312). Moreover, circulating levels of TNF, TNFRI, TNFRII, MMP-3 and TIMP-1 were independent predictors of patient

mortality (309,311,313-316). These clinical studies suggest that cytokines and MMPs may be used as biomarkers for the prognosis of HF. This article has also identified distinct differences in the circulating levels of cytokines and MMPs in DHF and SHF phenotypes. Generally, SHF patients have greater levels of circulating cytokines (TNF, TNFRI and IL-10) and MMPs (MMP-1 and -2) than DHF patients (310,317,318). Results from these initial clinical studies comparing DHF with SHF patients suggest that prospective HF clinical studies should account for differences in HF phenotypes. The clinical need to refine diagnosis, patient stratification, as well as therapeutic monitoring has sparked the continuous search for biomarkers as useful tools in heart failure management (180).

## Novel Biomarkers for Atrial Fibrilation

It is anticipated that over the next 4 decades, the prevalence of atrial fibrillation (AF) will increase dramatically owing to an aging population, improved therapies, and longer survival with heart disease (319,320). AF is associated with higher rates of stroke and hospitalization (321,322), diminished quality of life (323), and significant mortality (324). The identification of risk factors for developing AF is an important epidemiological task with potential implications for public health (325,326).

Well-established clinical risk factors for AF other than age and sex are body mass index, hypertension, and cardiovascular disease, including valvular disease and heart failure (327-329); however, these risk factors do not explain all cases of AF, which suggests a need for improvement in risk prediction and understanding of the pathophysiology of AF (330). Blood and urinary biomarkers are potential tools to enhance AF risk prediction and to provide insights into the pathophysiology of the disease. On the basis of biological plausibility and prior reports, biomarkers were chosen to represent distinct pathophysiological pathways, including inflammation (C-reactive protein [CRP] and fibrinogen) (331,332), neurohormonal activation (B-type natriuretic peptide [BNP] and N-terminal proatrial natriuretic peptide [N-ANP]) (333,334) oxidative stress and endothelial dysfunction (homocysteine) (335), the renin-angiotensin-aldosterone system (renin and aldosterone) (334), thrombosis and endothelial function (D-dimer and plasminogen activator inhibitor type 1) (336,337), and microvascular damage (urinary albumin excretion) (338).

BNP is a predictor of incident AF and improves risk stratification based on well-established clinical risk factors. Whether knowledge of BNP concentrations may be used to target individuals at risk of AF for more intensive monitoring or primary prevention requires further investigation (339).

A study on AF has demonstrated elevated levels of YKL-40 compared with healthy controls (340). In addition, YKL-40 was related to whether the patients had permanent or persisting AF, and thereby the burden of AF (341).

## Conclusion

Cardiac biomarkers have proved extremely valuable for diagnosis, risk stratification, and treatment of patients in the emergency setting. Novel studies are currently underway using biomarkers to predict long-term outcomes and mortality in patients with stable coronary heart disease. There is also much interest in the use of cardiac biomarkers to provide further risk stratification, especially in those patients who are at an intermediate risk per Framingham score.

To be clinically useful, a biomarker must provide incremental information that both adds to existing clinical findings and is useful in the clinical care of the patient. An ideal marker is one in which there is a specific, easily measurable increase that clearly aligns with a predictable outcome be it evidence of ischemia, inflammation, myocardial necrosis, plaque rupture, plaque destabilization, or heart failure. It remains a major challenge for researchers and clinicians to show whether newer and future biomarkers are useful for guiding specific treatment algorithms.

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