

Multimarker approach in cardiovascular risk prediction

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Abstract. Various biomarkers express different pathways and pathophysiologic mechanisms of cardiovascular disease, such as inflammation, oxidative stress, myocardial injury, activation of the neurohormonal pathways, myocardial stress and renal function. Current thinking supports the notion that the combination of these biomarkers could increase their diagnostic and prognostic value. The multimarker approach offers benefits since it increases the diagnostic and prognostic information and may help in the design of a strategy for prevention or management of cardiovascular diseases. The purpose of the current review is to describe the characteristics of promising biomarkers which have shown an important additive value in the assessment of cardiovascular risk. Also, an extended reference is made regarding studies that address the prognostic value of multimarker models in the settings of primary prevention of cardiovascular disease and secondary prevention for patients with acute coronary syndromes, chronic coronary artery disease and heart failure.

Keywords: Multimarker, biomarkers, cardiovascular risk, coronary artery disease, heart failure

Abbreviations and acronyms

ACS:	Acute Coronary Syndrome
ADM:	Adrenomedullin
BNP:	Brain Natriuretic Peptide
CAD:	Coronary Artery Disease
CRP:	C-Reactive Protein
cTnT, cTnI:	Cardiac Troponins T and I
ET-1:	Endothelin-1
IL-1b, IL-6, IL-10:	Interleukin 1b, Interleukin-6, Interleukin-10
Lp-PLA ₂ :	Lipoprotein-associated Phospholipase A ₂
MCSF:	Monocyte/Macrophage Colony Stimulating Factor
MPO:	Myeloperoxidase

MR-pro-ADM:	Midregional pro-Adrenomedullin
NT-Pro-BNP:	Amino-terminal pro Brain Natriuretic Peptide
OPG:	Osteoprotegerin
TGF β -1:	Transforming Growth Factor β -1
TNF- α :	Tumor Necrosis Factor α
WBC:	White Blood Cell Count
WBCHO:	Whole Blood Choline

1. Introduction

Circulating biomarkers have long since become a daily tool for the clinician in the diagnosis, management and prognosis of cardiovascular disease. In this context, novel biomarkers are continuously being developed in order to aid the physician in clinical decision and to enlighten pathophysiological paths associated with the pathogenesis of cardiovascular diseases and offer new targets for treatment. Different mechanisms, such as inflammation, oxidative stress, myocardial in-

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jury and cell apoptosis, activation of the neurohormonal pathways, elevation of intracardiac pressures and renal function, play important role in the pathogenesis of cardiovascular disease. Understanding of the above mechanisms may help in predicting the course of an acute cardiovascular event or the prognosis of a chronic ongoing disease process, and also aid in the design of a therapeutic strategy. It has been proposed that a multimarker approach to cardiovascular disease offers benefits in the assessment of cardiovascular risk, both for primary and secondary risk stratification. The purpose of the current review is: a) to describe the characteristics of promising biomarkers which have shown an important additive value in the assessment of cardiovascular risk, b) to review studies addressing the prognostic value of multimarker models in the settings of primary and secondary prevention of cardiovascular disease.

1.1. Biomarkers of inflammation

Inflammation is considered one of the major factors contributing to the pathogenesis of atherosclerosis [1]. A triggering effect, usually increased concentration of lipoproteins, but also shear stress forces, increased oxidative stress, angiotensin II, infections, initiates the atherogenic process. After the initial insult/injury of the vascular endothelium the process of atherosclerosis is perpetuated via inflammatory mechanisms, which include the release of proinflammatory cytokines, such as monocyte/macrophage colony stimulating factor (MCSF), interleukin 1b (IL-1b) and interleukin-6 (IL-6). These cytokines induce monocyte/macrophage activation, uptake of low density lipoprotein (LDL) and formation of foam cells which form the core of the atherosclerotic plaque. MCSF promotes the release of other cytokines such as IL-1b and IL-6 and finally leads to CRP production by the liver [2]. Furthermore, MCSF induces the production of monocyte chemoattractant protein-1 (MCP-1), which leads to leukocyte adhesion and migration into the vascular wall, and tissue factor (TF) related to plaque instability and thrombosis. Other cytokines such as TGF-1b have an anti-inflammatory/atheroprotective action by strengthening the fibrous cap over the atherosclerotic plaque.

The White Blood Cell Count (WBC) is the most easily obtained marker of inflammation. A high WBC count has been associated with increased cardiovascular mortality and incidence of coronary artery disease (CAD) in apparently healthy adults. In the setting of a known cardiovascular disease, WBC count seems to

correlate with increased risk for complications both in stable CAD (CAPRIE trial) [3] and acute coronary syndromes (ACS). In ACS, WBC count seems to have an additive prognostic value to that of troponin regarding the extent of CAD, TIMI flow and myocardial perfusion grade during angiography and mortality rate [4].

C-Reactive Protein (CRP) is the final end product of various inflammatory processes in atherosclerosis and acute coronary syndromes. It may reflect the cumulative effect of local vascular inflammation, but it may also have a direct proinflammatory effect on the endothelium by increasing the proinflammatory iNOS (nitric oxide synthetase) and reducing the atheroprotective eNOS. Its prognostic value has been shown for both primary and secondary prevention of coronary artery disease. There is evidence to support the additive value of CRP to that of traditional risk factors in the assessment of cardiovascular risk in apparently healthy adults [5,6], especially regarding values of the very low (< 0.5 mg/L) and very high range (> 10 mg/L) [7]. In the setting of an ACS, CRP has an additive predictive value to that of troponin [8] and it has been shown to predict long term survival regardless of LDL levels [9,10]. In acute and chronic heart failure CRP is an independent predictor of adverse outcomes [11].

The levels of Monocyte/macrophage colony-stimulating Factor (MCSF) have been found to be elevated in both stable CAD and acute coronary syndromes. MCSF is directly related to myocardial ischemia during effort and to the angiographic extend of coronary lesions [12]. Furthermore, levels of MCSF were found to have predictive value regarding short-term and long-term cardiovascular events in these situations, independently of traditional risk factors and CRP levels [13]. In acute coronary syndromes MCSF is a predictor of in-hospital mortality and short term survival. In chronic coronary artery disease increased levels of MCSF (> 800 pg/mL) and CRP (> 2.5 mg/L) were associated with a greater absolute and relative risk for future cardiovascular events, CRP, however, predicts cardiovascular events at a longer follow up period.

Tumor Necrosis Factor α (TNF- α) levels are elevated in patients with heart failure [14]. By activating matrix metalloproteinases TNF- α causes left ventricular dilatation and dysfunction. Moreover, as far as coronary artery disease is concerned, TNF- α and CRP retain their prognostic value independently of each other. However, TNF- α predicts short-term cardiovascular events in contrast to CRP which provides prognostic information for long-term outcomes [15]. Osteoprotegerin (OPG) is a member of the tumor necrosis

factor receptor family. OPG levels correlate with the severity of coronary artery disease [16] and it has been found to be an independent risk factor for cardiovascular events [17].

Interleukin-6 (IL-6) levels are found to be increased in stable and acute coronary syndromes [18]. IL-6 has a negative inotropic action [19] and its levels are also associated with elevated intracardiac pressures in patients with ischemic cardiomyopathy [20]. IL-6 was found to be increased in response to reversible ischemia during dobutamine stress echo and was related to the extent of new wall motion abnormalities at peak stress [21]. Thus, an increase of IL-6 at peak stress was a marker of reversible ischemia. Furthermore, increased IL-6 levels during recovery were related with a prolongation of the time to recovery of the new wall motion abnormalities observed at peak dobutamine stress echocardiography. This finding suggests that IL-6 may contribute to left ventricular dysfunction and may serve as predictor of the development of heart failure in patients with CAD. Indeed, changes of IL-6 during dobutamine stress echocardiography had an incremental value over wall motion abnormalities for the prediction of adverse outcome in CAD patients during a 6 year of follow-up [22].

Lipoprotein-associated Phospholipase A₂ (Lp-PLA₂) is produced by inflammatory cells that are involved in the process of atherogenesis and is detected in human atherosclerotic lesions. Elevated Lp-PLA₂ levels are associated with an increased risk of cardiovascular events [23] and adverse cardiovascular events in patients with coronary artery disease independently of traditional risk factors and CRP levels [24]. Pharmaceutical interventions to reduce Lp-PLA₂ activity may result in additional anti-inflammatory effect in patients with atherosclerosis, as was shown for darapladib administration and its effect on reducing high sensitivity CRP and IL-6 levels [25].

There are cytokines whose actions in innate and adaptive immunity have an anti-inflammatory effect, and are thus considered to be atheroprotective. Interleukin-10 (IL-10) is secreted from activated macrophages and forces CD4⁺ cells to differentiate into Th2, a response which is considered atheroprotective [26], in contrast to IL-12 which promotes the atherogenic Th1 response. Similarly to IL-10, transforming growth factor β -1 (TGF β -1) has an atheroprotective effect by inhibiting T-cell mediated immunity [27]. TGF β -1 also inhibits leucocyte adhesion molecules and MCP-1 expression, as well as smooth muscle cell differentiation, proliferation and migra-

tion [28]. TGF- β stimulates collagen production by smooth muscle cells leading to strengthening of the fibrous cap over the atheromatic plaque [29], and protecting from plaque instability and rupture.

1.2. Biomarkers of oxidative stress

Increased oxidative stress causes endothelial dysfunction, contributes to atherogenesis and leads to progression of heart failure through cell apoptosis and necrosis. Inflammation is tightly linked to oxidative stress status since various cytokines, such as interleukin-1, promote the release of superoxide anion. IL-1 also causes the release of NO⁻ which reacts with the superoxide anion to form the peroxynitrite, leading to the formation of nitrotyrosine, a marker of nitrosative stress [30]. Nitrooxidative stress also has a deleterious effect on endothelial function and is responsible for abnormal coronary vasoactivity in experimental models.

Myeloperoxidase (MPO) is a hemoprotein expressed in neutrophils and secreted during their activation. It causes oxidative transformation of LDL particles and thus plays a role in the initiation and progression of atherosclerosis and cardiovascular disease. MPO has potent proinflammatory properties and may contribute to tissue injury. Plasma myeloperoxidase serves as an indirect marker of oxidative stress. MPO levels have been associated with the risk of coronary artery disease independently of other risk factors [31]. Its levels were found increased in patients with coronary artery disease [32] and it is proposed to have prognostic value in acute coronary syndromes [33]. MPO levels also correlate with the severity of heart failure [34] and have prognostic value for the survival of these patients.

1.3. Biomarkers of myocardial injury

Cardiac troponins T and I (cTnT, cTnI) are highly specific and sensitive markers of myocardial necrosis. It has been shown that cTn measurement as a diagnostic tool is more useful than older biomarkers of myocardial necrosis (CK-MB, myoglobin). Even mild increase in troponin levels is of prognostic value in the setting of an acute coronary syndrome [35].

Other causes than ischemia may increase the levels of cardiac troponins. cTn may be increased in patients with heart failure without ischemia [36]. Myocardial injury and myocyte cell death can occur in heart failure due to elevated intracardiac pressures, inflammation or increased oxidative stress, and this fact, reflected on troponin levels, correlates with the severity of heart

failure and its adverse effects. A level of 0.04 ng/mL was found to be the cutoff point for cTnI regarding survival in patients with advanced heart failure after adjustment for other predictors [37]. Hudson et al. reported that cTnT levels of more than 0.02 ng/mL were associated with a 4-fold risk for death in patients with chronic heart failure [38]. In acute decompensated heart failure the levels of cardiac troponin may be used as predictors of outcome [39]. As more sensitive assays for the detection of troponin levels are being developed, its role in the prognosis of patients with heart failure will become more important in the future.

1.4. Biomarkers of increased myocardial stress – elevated intracardiac pressures

The natriuretic peptides, brain natriuretic peptide (BNP) and amino-terminal pro brain natriuretic peptide (NT-Pro-BNP), are produced by the prohormone BNP which is released as a response to increased wall tension, dilatation or hypertrophy of the ventricles [40]. BNP is a bioactive peptide which causes arterial vasodilation, diuresis and natriuresis, whereas NT-Pro-BNP is inactive. However, NT-Pro-BNP has a longer biologic half-time period than BNP (approximately 120 minutes), a fact that makes its levels useful in the diagnosis of heart failure. As a diagnostic-screening tool, the cut-off levels of BNP and NT-Pro-BNP were estimated at 100 pg/mL and 340 pg/mL respectively, bearing in mind the limitations of measurements in special populations (i.e. the elderly, patients with renal insufficiency, obese patients, females). The levels of BNP correlate with the in-hospital mortality of patients with acutely decompensated heart failure [41] and can be used as a prognostic marker independently of other variables. In chronic heart failure, BNP also has prognostic value, and it can be used for the risk stratification of these patients [42]. Recent studies have proposed that BNP should be used as a guide to intensify therapy in heart failure patients. In the STARS-BNP trial BNP-guided therapy was superior in terms of survival compared to therapy guided by clinical criteria [43]. Logeart et al. proposed that predischarge BNP levels are a strong predictor for adverse outcomes and readmission of patients with decompensated heart failure [44].

Adrenomedullin (ADM) is produced as a response to increased intracardiac pressures or volume overload and causes vasodilation and natriuresis [45]. Its levels are higher in patients with heart failure and correlate with the severity of the disease [46]. Midregional pro-adrenomedullin (MR-pro-ADM) is a more stable

molecule than adrenomedullin itself and can be measured more easily.

Increased levels of ST2, a member of the interleukin-1 receptor family, were found in patients with severe heart failure. ST2 levels are strong predictors of mortality both in patients with acute coronary events [47] and heart failure [48]. It was recently reported that pharmaceutical inhibition of IL-1 in patients with rheumatoid arthritis by anakinra, an IL-1a receptor antagonist, improves vascular and left ventricular function and is associated with reduction of nitrooxidative stress and endothelin [49]. The same medication (anakinra) was found to ameliorate the remodelling process of the left ventricle by inhibiting cardiomyocyte apoptosis in experimental animal models with myocardial infarction [50].

1.5. Biomarkers of neurohormonal activation

The activation of the sympathetic nervous system and renin-angiotensin-aldosterone system (RAAS) has been tightly linked to the pathogenesis and progression of heart failure [51]. Plasma levels of norepinephrine are an independent predictor of mortality in patients suffering from heart failure and in patients with left ventricular systolic dysfunction. The levels of endothelin-1 (ET-1) and big endothelin-1 correlate with the level of pulmonary artery pressure [52], and the severity and mortality of heart failure [53].

The activation of the RAAS is also prominent in acute coronary syndromes and leads to adverse cardiovascular effects via collagen synthesis and fibrosis. Reversal of this procedure by spironolactone, an inhibitor of aldosterone, reduces adverse left ventricular remodeling [54].

1.6. Biomarkers of renal dysfunction

Renal function is of pivotal importance in the assessment of cardiovascular events. Creatinine clearance is of additional prognostic value in the setting of an acute coronary event [55]. Cystatin C is a biomarker of renal function less influenced by gender, age and muscle mass than serum creatinine. Cystatin C correlates with the glomerular filtration rate which is generally accepted as an overall index of renal function. Cystatin C was found to be associated with peripheral atherosclerotic disease [56] and to predict the development of heart failure in the elderly [57]. Significant correlations were found between cystatin C values and other markers of cardiovascular disease, including CRP, in

elderly men [58]. There are studies suggesting a prognostic role for cystatin C in heart failure [59] and acute coronary syndromes [60].

Microalbuminuria has been documented as a risk factor for future cardiovascular events in hypertensive patients. For every 10-fold increase in UACR (urine albumin-creatinine ratio), hazard ratios in nondiabetic patients increased as follows: cardiovascular mortality, by 97.7%; all-cause mortality, by 75.2%; stroke, by 51.0%; and myocardial infarction, by 45% [61]. In patients with type 2 diabetes microalbuminuria and macroalbuminuria are considered risk factors for future cardiovascular events [62]. A significant percentage of patients with systolic heart failure without hypertension or diabetes also demonstrate microalbuminuria [63]. Ingelsson et al. have reported that microalbuminuria (defined as urinary albumin excretion rate of more than 4.7 $\mu\text{g}/\text{min}$) is a marker of subclinical cardiovascular damage which may predispose for future development of heart failure in the elderly (hazard ratio: 1.49) [64].

2. Multimarker risk prediction in various settings

2.1. Primary prevention

Markers of inflammation have been used in the general population as predictors of cardiovascular disease, either on their own or in various combinations. White blood cell (WBC) count was shown to be a predictor of cardiovascular events and all-cause mortality in postmenopausal women, independently of CRP levels. Women in the fourth quartile of WBC count (6700–15000 cells/L) had over a 2-fold elevated risk for coronary heart disease death (Hazard Ratio: 2.36), compared to women in the first quartile, after adjustment for known risk factors [65]. In the ABC study IL-6, CRP and TNF- α were examined regarding the incidence of cardiovascular events in the elderly. The three biomarkers were independently associated with the risk for developing heart failure and the greatest risk was found when all three biomarker measurements were in the top quartile [66]. Vasan et al. also reported that CRP, TNF- α and IL-6 levels could have prognostic value for the future development of heart failure in the elderly population. The combination of CRP with TNF- α seems to identify asymptomatic people for the risk of future development of heart failure in the Framingham Heart Study. Participants with elevated CRP levels ($> 5 \text{ mg}/\text{dL}$) had a 3-fold increase of heart failure risk, and participants with increased levels of all three

biomarkers had a 4-fold increase of risk [67]. The predictive value of IL-6 has been evaluated in several studies. It has been shown to be a risk factor for cardiovascular disease in apparently healthy men, independent of CRP [68]. However, IL-6's value as a prognostic marker in patients with acute coronary syndromes has not been established yet [69] and further studies are needed. On the other hand, CRP, IL-6 and other novel biomarkers were found to provide little additional prognostic information in a model including traditional risk factors in the ARIC study [70]. Ridker et al. in the recently published results of the JUPITER study, report that the use of hs-CRP levels as a guide to statin therapy in people without hyperlipidemia reduces the risk of adverse cardiovascular events in this apparently healthy population, suggesting an additive prognostic role of CRP to that of the already established role of LDL [71].

As far as markers of myocardial stress are concerned, the natriuretic peptides are the markers mostly studied. NT-Pro BNP was found to predict cardiovascular risk in nonhospitalised individuals aged 50–89 years [72]. In this study NT-pro-BNP was found to be a stronger biomarker in the prediction of cardiovascular events and death (Hazard Ratio:1.96) than CRP (Hazard Ratio:1.46) and urinary albumin/creatinine ratio (Hazard Ratio:1.88). In patients with other comorbidities such as hypertension, diabetes mellitus and asymptomatic coronary artery disease the value of BNP in predicting the risk for developing heart failure is also important. BNP may also be used to screen for cardiotoxic effects associated with the administration of certain medications [73].

In the HOPE study NT-Pro BNP was compared to traditional risk factors and other inflammatory biomarkers and it was found to be of greater value in the prediction of clinical outcome (myocardial infarction, stroke, cardiovascular death) during 4.5 years of follow-up than the 9 inflammatory biomarkers that were measured (hazard ratio for NT-Pro-BNP: 1.72 per increment SD, 95% CI:1.39–2.12, $p < 0.0001$) [74]. The result could be attributed to the significant day-to-day variability of inflammatory biomarker levels leading to misclassification of subjects, as was shown for CRP [75]. This can be rectified in part by the combination of more than one inflammatory biomarkers, as was done in the ABC [64] and FINRISK [13] studies.

CRP and NT-Pro-BNP may improve risk stratification of patients with chronic renal disease who present with increased levels of troponin. Patients with end stage renal disease and increased levels of cTnT and

CRP had an increased all-cause mortality risk (HR:3 and HR:1.8 in the fourth quartile for cTnT and CRP respectively), during a mean period of 4 years [76]. In another study, Apple et al. found that hs-CRP, cTnI and cTnT have additive prognostic value for all cause death during a 2 year follow-up period in patients with end stage renal disease. Tertile analysis for NT-Pro-BNP also demonstrated prognostic value [77].

Wang et al. measured 10 biomarkers (C-reactive protein, BNP, NT-Pro-BNP, aldosterone, renin, fibrinogen, d-dimers, plasminogen-activator inhibitor type 1, homocysteine, urinary albumin-to-creatinine ratio) in a population of the Framingham Heart Study (3209 participants). During a median follow-up period of 7.4 years 207 participants died and 169 had a first major cardiovascular event (fatal or non-fatal myocardial infarction, prolonged angina, heart failure, stroke). Of all examined biomarkers, BNP, CRP, urinary albumin-to-creatinine ratio, homocysteine and renin levels added incremental information for the prediction of the overall mortality in this population. BNP and urinary albumin-to-creatinine ratio showed the highest incremental predictive value for major cardiovascular events (adjusted HR:1.25 per 1 SD increment in the log values for BNP and HR:1.20 per 1 SD for urinary albumin-to-creatinine ratio). However, the use of multimarker scores was found to add only moderately to standard risk factors for risk assessment of individual persons (C statistic 0.77 with all predictors compared to 0.76 with age, sex and conventional risk factors as predictors) [78].

In a study by Zethelius et al., four biomarkers were measured (NT-Pro-BNP, cTnI, CRP, Cystatin-C) in the elderly (1135 participants, mean age at baseline 71 years) and there was a follow-up period of 10 years, during which 315 deaths from both cardiac and non-cardiac causes were recorded. This multimarker strategy was found to add prognostic information to that of established risk factors for cardiovascular disease. The participants with elevated levels of two, three or all four biomarkers had a 3-, 7- or 16-fold risk for cardiovascular death respectively. The authors concluded that the incorporation of a combination of biomarkers that reflect myocardial damage, renal function, myocardial function and inflammation to a model with established risk factors improved risk stratification for cardiovascular mortality in the elderly population. This finding was attributed in part to the older age of their study population compared to previous studies, but also to the proper selection of those biomarkers that reflect independent pathophysiological mechanisms affecting outcome [79].

2.2. Secondary prevention in

2.2.1. Coronary artery disease

Acute Coronary Syndromes (ACS)

In acute coronary syndromes, white blood cell count seems to have an additive predictive value to that of troponin regarding the extent of CAD, TIMI flow and myocardial perfusion grade during angiography, and mortality rate. The combined use of CRP and WBC was proposed to stratify patients with ACS across an eightfold gradation of 6-month mortality risk [2]. In unstable angina M-CSF has an additive prognostic value to that of CRP, independently of other traditional risk factors. As mentioned before, these biomarkers predict adverse cardiovascular events at a different stage in the depth of time, a fact that probably correlates with the production of these cytokines at different stages of the atherosclerotic process [11].

The addition of MCP-1 measurement to CRP and BNP levels provides additive prognostic value for adverse events (death, stroke, myocardial infarction, heart failure) in patients with acute coronary syndromes in the A to Z Study. A value of MCP-1 > 238 pg/ml was independently associated with mortality at 4 months of follow-up (HR: 1.76) [80]. In TACTICS-TIMI 18, patients with an ACS were found to have a 2.1-, 3.1, and 3.7-fold increase in the risk of death, myocardial infarction or heart failure in six months depending on the number of elevated biomarkers (CRP, BNP and cTnI), after adjustment for known clinical predictors of outcome [81]. Also, CRP has been associated with IL-6, TNF- α and fibrinogen in the setting of an acute coronary event [82], and cardiac mortality was found to be 6-fold higher in patients in the highest tertile of CRP and IL-6 and 3-fold higher in patients in the highest tertile of fibrinogen and TNF- α compared to the lower tertiles respectively.

In The TACTICS-TIMI-18 study, women presenting with ACS with elevated CRP and BNP levels were found to benefit from early revascularization therapy even if cardiac troponin levels were normal [83]. This finding suggests that BNP and CRP may determine risk and thus, guide treatment in patients with normal troponin levels. Regarding other markers of myocardial necrosis, however, troponin alone was shown to have the same prognostic value as a multimarker approach including myoglobin and CK-MB, according to Eggers et al. [84]. In another study of patients with ACS, the combination of cTnT and BNP levels was found of better prognostic value for a composite of death, myocardial infarction, unstable angina and the need for revas-

Table 1
Biomarkers in cardiovascular disease

<i>Biomarkers of Inflammation</i>
White Blood Cell Count (WBC)
C-Reactive Protein (CRP)
Macrophage/Monocyte Colony Stimulating Factor (MCSF)
Monocyte Chemoattractant Protein-1 (MCP-1)
Interleukins 1, 1b, 6, 10 (IL-1, IL-1b, IL-6, IL-10)
Transforming Growth Factor β 1 (TGF- β 1)
Tumor Necrosis Factor α (TNF- α)
Osteoprotegerin (OPG)
Lipoprotein-associated Phospholipase A ₂ (Lp-PLA ₂)
<i>Biomarkers of Oxidative and Nitroxidative Stress</i>
Nitrotyrosine
Myeloperoxidase (MPO)
<i>Biomarkers of Myocardial Injury and Ischemia</i>
Cardiac Troponins (cTnI, cTnT)
<i>Biomarkers of Myocardial stress</i>
Natriuretic Peptides (BNP, NT-Pro-BNP)
Interleukin-1 (IL-1)
Interleukin-6 (IL-6)
ST-2
Adrenomedullin (ADM), Midregional Pro Adrenomedullin (MR-Pro-ADM)
<i>Biomarkers of Neurohormonal Activation</i>
Norepinephrine
Endothelin-1 (ET-1) and Big Endothelin-1
<i>Biomarkers of Renal Function</i>
Creatinine Clearance
Microalbuminuria
Cystatin C

cularization procedures within 30 days than each marker alone. The combination of cTnT and BNP levels showed a sensitivity and relative risk of 78.6% (66.2–91) and 4.7 (2.3–9.5) respectively for adverse events during follow-up. Moreover, the prognostic value of the combination of cTnT and BNP levels was better than that of the combination of cTnT and CRP levels. Furthermore, the addition of high sensitivity CRP measurement in the panel of cTnT and BNP levels did not add any further prognostic information [85].

Osteoprotegerin (OPG) and BNP levels are increased in acute coronary syndromes. In a recent report by Palazzuoli et al., OPG levels were increased in patients with stable angina compared to controls, however, patients with non ST-elevation acute coronary syndromes had higher OPG levels compared to patients with stable angina (11.8 ± 7.1 pmol/l, $p < 0.001$). The levels of OPG correlated with the number of coronary plaques, whereas BNP correlated with the number of the affected vessels [86]. The findings of the above studies suggest that OPG and BNP may provide complementary information about the atheromatic load and plaque instability in patients with CAD.

In acute coronary syndromes the prognostic value of BNP may be enhanced when combined with the measurements of midregional pro-adrenomedullin (MR-

pro-ADM). Both biomarker measurements were strong predictors of cardiovascular death or heart failure 3–5 days after the onset of symptoms of the acute event, and MR-pro-ADM provided further risk stratification in patients with increased NT-Pro-BNP levels [87].

Also, the state of renal function, as estimated by creatinine clearance, may further enhance the predictive value of BNP and troponin levels in the risk assessment of patients with acute coronary syndromes. Suwaidi et al. described the prognostic value of renal function for death and myocardial infarction in patients with an acute coronary event at 30- and 180-days. Creatinine clearance was independently associated with risk of mortality (hazard ratio 0.79 in the ST-elevation group and 0.81 in the non-ST-elevation group) and with risk of mortality/MI (hazard ratio 0.93) in the non-ST-Elevation group at 180 days [88].

Mockel et al. evaluated possible combinations between various biomarkers (cardiac troponin I, NT-Pro-BNP, high sensitivity C-reactive protein, placental growth factor, Lp-PLA₂, D-dimers, whole blood choline and plasma choline) in patients presenting with an ACS in the emergency department. They found that the combination of NT-Pro-BNP, whole blood choline (WBCHO) and LpPL-A₂ was optimal for risk stratification and superior to other combinations of biomarkers.

Table 2
Multiple biomarker combinations

Biomarkers studied	Associations with outcome independent of established risk factors
<i>Primary Prevention</i>	
CRP, IL-6 (Ridker et al.) [68]	CRP, IL-6
CRP, TNF- α , IL-6 (Tuomisto et al.) [15]	CRP, TNF- α
CRP, IL-6, TNF- α (Cesari et al. [66], Vasani et al. [67])	CRP, IL-6, TNF- α
NT-Pro-BNP, cTn, CRP, Cystatin C (Zethelius et al.) [79]	NT-Pro-BNP, cTn, CRP, Cystatin C
<i>Secondary Prevention</i>	
1. <i>Coronary Artery Disease</i>	
cTn, WBC (Sabatine et al.) [4]	cTn, WBC
CRP, MCSF (Ikonomidis et al.) [13]	CRP, MCSF
CRP, MCP-1, BNP (de Lemos et al.) [80]	CRP, MCP-1, BNP
CRP, BNP, cTnI (Sabatine et al.) [81]	CRP, BNP, cTnI
CRP, IL-6, TNF- α , Fibrinogen (Koukkunen et al.) [82]	CRP, IL-6, TNF- α , Fibrinogen
CRP, cTn, NT-Pro-BNP, CK-MB, Myoglobin (Cameron et al.) [85]	cTn, NT-Pro-BNP
CRP, Lp-PLA ₂ (Sabatine et al.) [95]	CRP, Lp-PLA ₂
NT-Pro-BNP, OPG (Palazzuoli et al.) [86]	NT-Pro-BNP, OPG
BNP, MR-Pro-ADM (Khan et al.) [87]	BNP, MR-Pro-ADM
NT-Pro-BNP, IL-6, CRP, Fibrinogen, Cystatin C, Albuminuria (Shlipak et al.) [96]	NT-Pro-BNP, CRP, Albuminuria
cTnI, NT-Pro-BNP, CRP, WBCHO, PLCHO, LpPLA ₂ , Placental Growth Factor, D-Dimers (Mockel et al.) [89]	NT-Pro-BNP, WBCHO, LpPLA ₂
Cystatin C, NT-Pro-BNP, Lp-PLA ₂ (Koenig et al.) [94]	Cystatin C, NT-Pro-BNP, Lp-PLA ₂
cTn, BNP, MPO, CD40 (Morrow et al.) [91]	cTn, BNP, MPO
CRP, γ GT, Glucose (Emdin et al.) [93]	CRP, γ GT, Glucose
2. <i>Heart Failure</i>	
BNP, cTn (Fonarow et al.) [101,102]	BNP, cTn
BNP, cTn, CRP (Yin et al.) [104]	BNP, cTn, CRP

Increased or low concentrations of NT-Pro-BNP and WBCHO identified patients of very high or very low risk for major adverse cardiovascular events (cardiac death, non-fatal myocardial infarction, unstable angina, heart failure requiring admission, urgent PCI and CABG) during 42 days of follow-up respectively [89].

Myeloperoxidase (MPO) levels correlate with the prevalence of cardiovascular disease and were found to predict early and late cardiovascular events after an ACS [90]. MPO levels add to the prognostic value of BNP and cTnI regarding short-term cardiovascular effects of patients with non-ST elevation ACS. Patients with elevated baseline MPO (> 884 pM) were at higher risk of non-fatal myocardial infarction or rehospitalization for ACS at 30 days (9.3 vs. 4.6%, $P < 0.001$). In contrast to MPO, soluble CD40 ligand levels were not associated with risk in this population [91].

Chronic Coronary Artery Disease (CAD)

Studies have shown that there is a synergistic value of MCSF and CRP regarding absolute and relative risk for future cardiovascular events during a 6 year follow-up in patients with chronic CAD [11]. However, it seems that MCSF is a better prognostic marker for short term outcome, whereas CRP predicts cardiovascular events at a longer period of time. This prob-

ably reflects cytokine production at different stages of the inflammatory process. Increased levels of TNF- α predict a shorter event-free interval, compared to CRP which predicts long-term cardiovascular events, a fact that suggests a role of CRP in more advanced stages of atherogenesis. Increased levels of CRP and IL-6 in combination to high MCSF or low TGF β -1 predict adverse outcomes in CAD patients independently of traditional risk factors [92]. In the context of the multimarker approach, older biomarkers are re-evaluated, as was done for gamma-glutamyltransferase (γ GT), which was found to have an additive prognostic value for mortality to other biomarkers, such as CRP, during a 3-year follow-up period in patients with angiographically documented coronary artery disease [93].

Koenig et al. estimated the incremental value of Lp-PLA₂ to a model including classical risk factors, renal function as assessed by cystatin C and hemodynamic stress as assessed by NT-Pro-BNP for cardiovascular events (death, non-fatal MI, stroke) in patients with CAD during 4 years of follow-up. They found that addition of cystatin C and NT-Pro-BNP measurement to the basic model improved its predictive accuracy (Area Under the Curve: 0.71 from 0.69) and when Lp-PLA₂ levels were added the AUC showed a small increase (0.73 from 0.71). In the multivariable analyses there

was a near 2-fold increased risk for future cardiovascular events in patients in the top two tertiles of Lp-PLA₂ mass compared to the lower tertile, after adjustment for markers of inflammation, renal dysfunction and hemodynamic stress [94]. In the PEACE trial, Sabatine et al. showed that elevated Lp-PLA₂ and hs-CRP levels were highly significant predictors of acute coronary syndromes in patients with stable coronary artery disease ($p < 0.005$ and 0.001 respectively), whereas only Lp-PLA₂ was a significant predictor for coronary revascularization during 4.8 years days of follow-up [95].

In the Heart and Soul Study the prognostic value of six biomarkers (NT-Pro-BNP, IL-6, CRP, fibrinogen, Cystatin C and albuminuria) was evaluated in patients with chronic coronary artery disease during an average of 3.5 years of follow up. After multivariable analysis including all biomarkers, NT-Pro-BNP, CRP, and albuminuria were found to retain their prognostic value for cardiovascular events (NT-Pro-BNP Hazard Ratio:1.88, albuminuria Hazard Ratio:1.63, and CRP Hazard Ratio:1.82). Adding all three biomarkers significantly increased the area under the receiver operator curve for the prediction of adverse outcome to 0.77 compared to 0.73 when each biomarker was used on its own [96].

2.3. Heart failure

Markers of inflammation are elevated in heart failure. It has been proposed that an initial precipitating event, such as an ischemic cardiac injury, leads to the initiation of inflammatory process via cytokine production, which in turn causes impairment of left ventricular function and accelerates the progression of heart failure [97]. TNF- α and interleukins 1,6, and 10 have been found to be elevated in patients with heart failure [98]. However, the usefulness of interleukins and TNF- α in a multi marker panel as predictive markers in heart failure has not, to our knowledge, been evaluated.

The prognostic value of various neurohormones has been evaluated in the Valsartan Heart Failure Trial (Val-HeFT). All the neurohormones in patients with advanced heart failure were significant markers of outcome. BNP was found to be the most powerful predictor of mortality and hospitalization followed by big endothelin-1, norepinephrine, endothelin-1, plasma renin activity, and aldosterone [99].

The natriuretic peptides (BNP and NT-Pro-BNP), biomarkers of elevated intracardiac pressures and myocardial stress, have been shown to add prognostic information about the severity and adverse outcomes in

heart failure patients. Supplementary prognostic information to that provided by the natriuretic peptides may be gathered by the measurement of the levels of midregional pro-adrenomedullin (MR-pro-ADM). Khan et al. report that MR-pro-ADM has an additive prognostic value to the levels of NT-Pro-BNP for the development of heart failure after an acute myocardial infarction [84]. However, MR-pro-ADM's predictive value in patients with known heart failure has not been evaluated.

ST2, an interleukin-1 receptor family member, has been studied in patients with heart failure (NYHA class III or IV) and baseline levels correlated with the levels of BNP, Pro-ANP and norepinephrine. Furthermore, after multivariate analysis the change in serum soluble ST2 levels remained significant predictor for mortality or need for heart transplantation, independently of BNP or Pro-ANP levels, suggesting an incremental value of ST2 to BNP or Pro-ANP [100].

Myocardial cell injury and necrosis, as expressed by elevated levels of cardiac troponins, is associated with the progression of heart failure and adverse effects of the disease, as is already well documented. Fonarow et al. have shown that heart failure patients with detectable cardiac troponin I and high BNP levels have a 12-fold increased mortality risk compared to those with both undetectable cardiac troponin I and lower BNP [101]. The investigators of the ADHERE study document that a level of BNP > 840 pg/mL and increased levels of troponin was associated with a greater mortality risk than BNP < 840 pg/mL and normal values of troponin (mortality rate 10.2% and 2.2% respectively), showing that admission BNP and cTn levels are significant, independent predictors of in-hospital mortality in acutely decompensated HF [102]. The measurement of high sensitivity troponin T (hsTnT) in patients with heart failure was evaluated by Latini et al., and it was shown that troponin T retains a prognostic value at previously undetectable concentrations. cTnT was associated with death and first hospitalization for heart failure (HR:2.08 and 1.55 respectively) and hsTnT was associated with mortality risk (HR:1.05, CI:1.04–1.07 for increments of 0.01 ng/mL) [103].

It has been proposed that a multimarker approach including markers of myocardial injury, hemodynamic stress, inflammation and renal function could provide further prognostication than each biomarker alone. Yin et al. propose that the combination of NT-Pro-BNP, cTnI and high sensitivity CRP (hsCRP) have an additive prognostic value to single biomarker measurement in patients with advanced heart failure during a median

follow-up period of 186 days. Patients with one, two or three elevated biomarkers had a 2.7- ($p = 0.125$), 8.6- ($p < 0.0001$) and 23.4- ($p < 0.0001$) fold increase in the risk of adverse events (cardiac death, requirement for heart transplantation or hospitalization for worsening of heart failure) respectively [104].

With the exception of BNP and cTn, little is known about the incremental predictive value of other biomarkers in the risk stratification of patients with heart failure. Several biomarkers of inflammation, myocardial stress, myocardial necrosis, oxidative stress and renal function could be evaluated in a multimarker panel in hope that they may improve risk assessment in heart failure patients and help optimize their treatment.

3. Conclusion

Various biomarkers express different pathways and pathophysiologic mechanisms of cardiovascular disease. The work in the field of biomarkers is ongoing and novel biomarkers are being developed and studied constantly. Current thinking supports the notion that the combination of these biomarkers could increase their diagnostic and prognostic value. Research towards this so-called multimarker approach is far from completed and new evidence comes to light as novel biomarkers are being examined. The multimarker approach offers benefits since it allows the study of cardiovascular disease through many different mechanisms, it increases the diagnostic and prognostic information and may help in the design of a strategy for prevention or management of cardiovascular diseases. However, the optimal combination of biomarkers has not been defined yet and further work in this field is needed in order to improve the risk stratification of patients with cardiovascular disease and help physicians optimize their management.

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