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Review · **Open Access** ·

Novel biomarkers for cardiovascular risk prediction

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Abstract

Cardiovascular disease (CVD) is the leading cause of death and disability worldwide. The primary prevention of CVD is dependent upon the ability to identify high-risk individuals long before the development of overt events. This highlights the need for accurate risk stratification. An increasing number of novel biomarkers have been identified to predict cardiovascular events. Biomarkers play a critical role in the definition, prognostication, and decision-making regarding the management of cardiovascular events. This review focuses on a variety of promising biomarkers that provide diagnostic and prognostic information. The myocardial tissue-specific biomarker cardiac troponin, high-sensitivity assays for cardiac troponin, and heart-type fatty acid binding proteinall help diagnose myocardial infarction (MI) in the early hours following symptoms. Inflammatory markers such as growth differentiation factor-15, high-sensitivity C-reactive protein, fibrinogen, and uric acid predict MI and death. Pregnancy-associated plasma protein A, myeloperoxidase, and matrix metalloproteinases predict the risk of acute coronary syndrome. Lipoprotein-associated phospholipase A2 and secretory phospholipase A2 predict incident and recurrent cardiovascular events. Finally, elevated natriuretic peptides, ST2, endothelin-1, mid-regional-pro-adrenomedullin, copeptin, and galectin-3 have all been well validated to predict death and heart failure following a MI and provide risk stratification information for heart failure. Rapidly developing new areas, such as assessment of micro-RNA, are also explored. All the biomarkers reflect different aspects of the development of atherosclerosis.

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1 Introduction

Cardiovascular disease (CVD) is the leading cause of death and disability worldwide.[1] Conventional risk factors for CVD, such as hypertension, diabetes mellitus, smoking, and hypercholesterolemia, have led to the development of risk prediction models and to major developments in therapy. However, up to 20% of patients with coronary disease have no traditional risk factors, and 40% have only one.[2] The implementation of such strategies in a cost-effective manner is restricted by the limited predictive value of the current risk-assessment models. In this review, we discuss ongoing novel risk biomarkers to enhance the current risk-stratification metrics for CVD and improve the selection of individuals for preventative strategies.

Biomarkers refer to a broad subcategory of quantifiable and reproducible characteristics of biological signs. In the broad sense, they are "a characteristic that is objectively measured and evaluated as an indication of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[3] Useful biomarkers must meet the following criteria: (1) accuracy: that is, the ability to identify individuals at risk; (2) reliability: that is, the stability of results when repeated; and (3) therapeutic impact with early intervention.[4]

We have, therefore, performed a systematic search on PubMed, Web of Science, and Scopus with no date restrictions and using the keywords "biomarker" and ''cardiovascular disease'' or ''acute coronary syndrome'' or "coronary artery disease" or ''myocardial infarction'' or ''heart failure''. We manually selected emerging biomarkers and those on the horizon in the categories of myocardial necrosis, inflammation, plaque instability, platelet activation, myocardial stress, neurohormonal activation and excluded those traditional proinflammatory molecules such as IL-6, TNFα and VCAM-1. The novel biomarkers indicating of various pathophysiological processes associated with cardiovascular disease were summarized in Table 1.

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Table 1. Biomarkers related to various pathophysiological processes associated with cardiovascular disease.

Biomarker	Overview
Myocardial necrosis	
cTn	Clinical studies support the relationship with CVD and AMI. A dynamic elevation of cardiac troponin above the 99th percentile of healthy individuals indicates AMI. However, conventional cTn assays is their low sensitivity at the time of AMI presentation.
hs-cTn	Several large multicenter studies have consistently shown hs-cTn assay increase the accuracy of AMI diagnosis, and it might be an excellent tool for risk stratification.
H-FABP	Data has shown that H-FABP is either superior to or adds incremental value to cTn in the early diagnosis of ACS. H-FABP could be a useful indicator for the early identification of high risk patients.
Inflammation	
HsCRP	Studies have confirmed an association of CRP and cardiovascular events independent of other cardiovascular risk factors. HsCRP that detects lower levels of CRP (< 5mg/L) could help detect high risk patients more early and accurately. However, the causal association is unknown.
GDF-15	Studies have shown that GDF-15 is a strong predictor of cardiovascular events and all cause death. Clinical trials suggest that GDF-15 is a potential tool for risk stratification.
Fibrinogen	Prospective studies support that elevated fibrinogen levels are associated with an increased risk of incident CVD. ESC guidelines allow fibrinogen measurement as a part of the risk assessment in patients with an unusual or moderate cardiovascular risk
UA	Recent studies have shown an independent positive association between UA and cardiovascular mortality. However, there is still conflicting evidence for the results.
Plaque instability	
PAPP-A	Observational studies suggests that circulating PAPP-A is a promising biomarker for risk stratification of ACS.
MPO	Prospective and cross-sectional studies addressed the role of MPO as a circulating inflammatory marker in CVD. However, its routine measurement is not recommended in clinical settings
MMPs	Studies have shown MMP-2, MMP-8, and MMP-9 have been recognized as proteases that contribute to plaque rupture and clinical events
Platelet activation	
Lp-PLA2	Although elevated Lp-PLA2 levels have been shown to be associated with an increased cardiovascular risk independent of other covariates, the overall incremental clinical utility of this biomarker remains unclear.
sPLA2	Observational studies have indicated that higher circulating sPLA2-IIA and sPLA2 levels are associated with an increased risk of cardiovascular events. However the clinical value is not clear.
sCD40L	Perspective studies have reported the prognostic value of sCD40L for detecting cardiovascular events. However, the results of some investigations are controversial.
Neurohormonal activation	
Copeptin	Studies have shown that copeptin could predict CAD development and cardiovascular mortality, while whether the heart contributes to its release is unknown.
MR-proADM	Studies showed that MR-proADM is a promising biomarker for risk prediction in patients with HF and for early atherosclerotic plaque development and subclinical CAD.
Myocardial stress	
NPs	In the current European guidelines, the NT-proBNP and MR-proANP are regarded as equal for the diagnosis of HF
ST2	The studies have confirmed the role of ST2 in cardiovascular risk stratification.
ET-1	Studies have shown CT-proET-1 was associated with cardiovascular death and HF independent of clinical variables.
Gal-3	Gal-3 was approved by FDA in 2010 as a new biomarker in the risk stratification of HF.
NRG-1	Studies have shown higher NRG-1 levels correlated with HF and CAD. However its use in clinical set as a risk factor needs further studies.
MicroRNAs	Several cardiac miRNAs are increased early after MI. However, their detection techniques are time consuming and their clinical benefits beside current diagnostic tools remain unclear.

ACS: acute coronary syndrome; AMI: acute myocardial infarction; CAD: coronary artery disease CVD: cardiovascular disease; cTn: cardiac troponin; ET-1: Endothelin-1; H-FABP: heart-type fatty acid binding protein; hs-cTn: high-sensitivity cardiac troponin; hsCRP: high-sensitivity C-reactive Protein; Gal3: galectin-3; GDF-15: growth-differentiation factor-15; Lp-PLA2: lipoprotein-associated phospholipase A2; miRNAs: microRNAs; MMPs: matrix metalloproteinases; MPO: myeloperoxidase; MR-proADM: mid-regional-pro-adrenomedullin; NPs: natriuretic peptides; NRG-1: Neuregulin-1; PAPP-A: pregnancy-associated plasma protein-A; sCD40L: soluble CD40 ligand; sPLA2: secretory phospholipase A2; UA: uric acid.

2 Biomarkers of myocardial injury

2.1 Cardiac troponin

Troponin is a complex of three globular contractile regulatory proteins (troponin T, I, and C) that reside in regular intervals in the thin filament of striated muscle that inhibits contraction by blocking the interaction of actin and myosin.[5] Cardiac troponin I (cTnI) and T (cTnT) are proteins that are unique to the heart and are specific and sensitive biomarkers of myocardial damage.[6] The cTnT and cTnI are different in skeletal and cardiac muscle, which allows for their use as a cardiac specific biomarker. The troponin C found in type 2 fibers of the skeletal muscle and the cardiac muscle are identical; therefore, it is difficult to be used as a cardiac specific marker.

In acute myocardial infarction (AMI), cTnI and cTnT are released from necrotic myocardium as both intact proteins and degradation products. The detection of cTn in peripheral blood indicates and quantifies cardiomyocyte damage. Cardiac troponins are more sensitive and specific markers of cardiomyocyte injury than creatine kinase (CK), its MB isoenzyme (CK-MB) and myoglobin. If the clinical presentation is compatible with myocardial ischemia, a dynamic elevation of cardiac troponin above the 99th percentile of healthy individuals indicates AMI.[7] However, a major limitation of conventional cTn assays is their low sensitivity at the time of AMI presentation, which is due to a delayed increase in circulating levels and requires serial sampling for 6–9 h in a significant number of patients.

2.2 High-sensitivity cardiac troponin (hs-cTn)

Technological advances have led to a refinement in cTn assays and have improved the ability to detect and quantify cardiomyocyte injury.[6] Recently, a newer generation of troponin assay with greater sensitivity has become available. The emergence of these hs-cTn assays has changed the role of cTn from a marker used only in the acute diagnosis of disease to a marker that assesses ongoing myocardial injury in stable patients and even seemingly healthy populations. Sensitive cTn and hs-cTn assays have two different features from conventional cTn assays: (1) the detection of cTn in a substantial number of healthy persons and (2) a more precise definition of "normal level" (the 99th percentile) with a more precise assay.[8]

In patients with AMI, levels of cardiac troponin rise rapidly, usually within 1 h if using high-sensitivity assays after symptom onset, and they remain elevated for a variable period of time[9]. Data from several large multicenter studies have consistently shown that sensitive cTn and hs-cTn assays increase the accuracy of AMI diagnosis at the time of presentation to the emergency department.[8,10,11] A recent study further broadens the association between high-sensitivity troponin and 5-year outcomes among patients with diabetes mellitus and stable coronary artery disease (CAD). This study showed a strong, consistent association between the baseline concentrations of circulating cTnT and the risk of all cause death, myocardial infarction (MI), stroke, and heart failure (HF) in patients with both type 2 diabetes and stable CAD. These results suggest that employing the hs-cTn assay for patients with diabetes and CAD is an excellent tool for risk stratification.[12]

2.3 Heart-type fatty acid binding protein (H-FABP)

Cytoplasmic FABP represent a family of transport proteins that allows for the transport of fatty acids through the membranes. FABP is tissue specific; thus, there are liver-type FABP (L-FABP), intestinal-type (I-FABP), brain-type FABP (B-FABP), and heart-type FABP (H-FABP).[13] H-FABP is a low molecular weight protein comprised of 132 amino acids and is involved in myocardial fatty-acid metabolism. It is found in abundance in cardiomyocytes and also in small quantities in the brain, kidney, and skeletal tissue, and its levels can increase in response to acute ischemic strokes and intense exercise. H-FABP is rapidly released into the cytosol early in AMI.

Recent studies have showed that H-FABP is either superior to or adds incremental value to troponin in the early diagnosis of acute coronary syndrome (ACS), as demonstrated by ROC analyses.[14,15] Kabekkodu, *et al.*[16] observed that among AMI patients presenting within four h of symptom onset, the sensitivity of H-FABP was 60%, which is significantly higher than that of cTnI (18.8%) and CK-MB (12.5%). However, the specificity was only 23.53%, which is less than that of cTnI (66.67%) and CK-MB (100%). During 4–12 h of symptom onset, the sensitivity of H-FABP was 86.96%, comparable to that of cTnI (90.9%) and CK-MB (77.3%) and the specificity was 60% in the 4–12 h group, comparable to that of cTnI (50%) and CK-MB (50%). Furthermore, the H-FABP level was increased in association with greater numbers of cardiovascular risk factors and was an independent risk factor for all-cause and cardiovascular death. Accordingly, H-FABP could be a useful indicator for the early identification of high risk patients in the general population.[17]

3 Biomarkers of inflammation

3.1 High-sensitivity C-reactive protein (hsCRP)

CRP is a member of the pentraxin family of innate immune response proteins. It is a nonspecific inflammatory

marker that has been extensively studied in CVD.[18] CRP itself mediates atherothrombosis.[19]

The Women's Health Study and the Physicians' Health Study, performed in healthy women and men, respectively, showed an association of CRP and cardiovascular events independent of other cardiovascular risk factors.[20,21] HsCRP that detects lower levels of CRP (< 5mg/L) stratifies patients into low, intermediate, and high risk, thus those classified as intermediate and high risk could benefit from aggressive therapy.[22] In a meta-analysis, encompassing more than 160,000 subjects with 1.3 million person-years of follow-up and nearly 28,000 incidents of cardiovascular events, each standard deviation increase in hsCRP (log-normalized) was associated with a relative risk increase of 1.37 for CAD (95% CI: 1.27–1.48) and 1.55 (95% CI: 1.37–1.76) for cardiovascular mortality.[23] Furthermore, in patients undergoing percutaneous coronary intervention (PCI), higher CRP levels at the time of the procedure are predictive for 10-year mortality and MI.[24] The European Society of Cardiology (ESC) guidelines also gives hsCRP a Class IIb recommendation, stating that hsCRP may be measured as part of refined risk assessment in patients with unusual or moderate cardiovascular risk profiles.[25] Thus, the interpretation of hsCRP results is straightforward: levels < 1 mg/L are desirable and reflect a low systemic inflammatory status and lower atherosclerotic risk; levels between 1 and 3 mg/L indicate moderate vascular risk; levels > 3 mg/L indicate higher vascular risk in the context of other risk factors and values that are > 10 mg/L may reflect a transient infectious process or other acute phase response, thus should be repeated within two to three weeks. Although it has direct association with cardiovascular events and recent investigations have confirmed CRP to be a predictor of cardiovascular events, hsCRP is unlikely to be a causal factor of CVD.[26–28]

3.2 Growth-differentiation factor-15 (GDF-15)

GDF-15, previously referred to as macrophage-inhibitorycytokine-1, is a divergent member of the transforming growth factor-β cytokine superfamily and is expressed by activated macrophages.[29] It is associated with cellular oxidative stress, ischemia, and strain; however, it is unknown whether GDF-15 is causally involved in the pathological process leading to CVD or has a cellular protective function.[30, 31] Kempf, *et al.*[32] monitored knockout mice and found that GDF-15 played a major role in controlling inflammatory cell recruitment by directly interfering with leukocyte integrin activation, thereby inhibiting leukocyte arrest and extravasation. The results suggest that GDF-15 acts as an inhibitor of leukocyte recruitment in the heart.

GDF-15 is a strong predictor of all-cause, cardiovascular, and non-cardiovascular mortality in community-dwelling elderly individuals, adding incremental value to traditional risk factors and CRP levels, thereby suggesting a fundamental role in the biological processes associated with aging.[33] A recent study has shown that temporal changes of GDF-15 concentrations improved risk prediction in an elderly population.[34] In acute heart failure (AHF) patients enrolled in the RELAX-AHF study, increased GDF-15 levels were associated with a greater likelihood of adverse outcomes.[35] The FRISC-II study, which randomized patients with non-ST segment elevated myocardial infarction (NSTEMI) to conservative and early invasive strategies, found that GDF-15 could predict death or recurrent MI in the conservative group but not in the invasive group, which suggests that GDF-15 improved patient selection for early invasive strategy.[36] The association of GDF-15 with CVD, such as ACS, stable CAD, and HF, makes it a novel promising biomarker for risk assessment, independent of other established risk biomarkers.[37] Studies about the cardiovascular risk stratification of GDF-15 are summarized in Table 2.[38–43] Wollert, *et al.*[44] reported two cut-offs for GDF-15. The value of 1200 ng/L was considered an optimal cut-off for presumably healthy individuals, and the value of 1800 ng/L was considered optimal in patients with Non ST-elevation acute coronary syndromes (NSTEACS) and for the purposes of risk stratification in ACS patients. However, GDF-15 is not specific for CVD and has been found to be elevated in a variety of malignancies (prostate, colon, glial). Nevertheless, promising results from clinical trials suggest that GDF-15 is a potential tool for risk stratification and therapeutic decision-making.

3.3 Fibrinogen

Fibrinogen was the first clotting factor, discovered and described in the first half of the nineteenth century.[45] Fibrinogen is an acute phase protein synthesized in the liver, and its circulating levels can exceed 7 mg/mL during acute inflammation. Furthermore, it is involved in platelet aggregation, endothelial injury, plasma viscosity, and plays a central role in the formation of thrombus.

Elevated fibrinogen levels are associated with an increased risk of incident CVD. The FSC study assessed the relationship of fibrinogen concentrations and the risk of both major vascular and non-vascular outcomes based on 154,211 individual participants' data without known CVD from 31 prospective studies.[46] The results showed that fibrinogen concentration was a risk factor for CAD, stroke, and mortality. In the ERFC study, Kaptoge, *et al.*[47] analyzed data from 53 prospective studies involving 246,669

Table 2. Studies using GDF-15 for cardiovascular risk stratification.

Study population	*n*	Endpoint	Thresholds	Hazard ratio
ALPS-AMI[38]	430	All-cause death, MI, stroke, or hospitalization due to congestive HF	< 1221 ng/L, ≥ 1221 ng/L	1.001
PLATO trial[39]	16,876	Cardiovascular death, spontaneous MI, and stroke	Quartile (< 1145 ng/L,1145–1550 ng/L,1550–2219 ng/L,> 2219 ng/L)	1.4
IABP-SHOCK II[40]	600	All-cause mortality	Median	1.88
Suspected AMI[41]	1247	All-cause death, AMI	< 1200 ng/L,1200–1800 ng/L, > 1800 ng/L	19.2, 20.1
NSTE-ACS[42]	1146	Deaths and nonfatal MI	Median	2.4
AtheroGene[43]	1781	Nonfatal MI, cardiovascular mortality	≥ 1499 ng/L	2.81, 2.67
PIVUS study[34]	1016	All-cause mortality	Median (1242 ng/L)	1.68

ACS: acute coronary syndrome; AMI: acute myocardial infarction; HF: heart failure; MI: myocardial infarction.

participants without a history of CVD, and it was found that the assessment of CRP or fibrinogen concentrations was associated with a significant improvement in the prediction of cardiovascular events. Assessment of the CRP or fibrinogen level in people at an intermediate risk for a cardiovascular event could help prevent one additional event over a period of 10 years for every 400 to 500 people screened.

Moreover, fibrinogen is composed of two sets of three polypeptide chains: Aα, Bβ, and γ and 8–15% of circulating fibrinogen in healthy individuals contains a γ chain (γA/γ′). Recently, a large prospective study showed a positive association of γ′ fibrinogen with incident CAD, ischemic stroke, peripheral artery disease, HF, and cardiovascular deaths.[48] This observation has suggested that γA/γ′ fibrinogen is a causal risk factor for CVD. Both *in vitro* and *in vivo* findings show that in patients with post-AMI, an overall imbalance in redox status and marked fibrinogen carbonylation is associated with altered clotting activity and susceptibility of plasmin-induced lysis.[49] These features may contribute to greater insight into the pathophysiology of fibrinogen in acute cardiovascular events. ESC guidelines on CVD prevention in clinical practice allow fibrinogen measurement as a part of the risk assessment in patients with an unusual or moderate cardiovascular risk but not in asymptomatic low-risk individuals.

3.4 Uric acid (UA)

UA is the end product of purine metabolism in humans. The inactivation of uricase and the increased levels of UA are thought to have provided evolutionary advantages by protecting against oxidative damage.[50] Elevated serum UA has been hypothesized to contribute to CVD development, even below the clinical threshold for hyperuricemia[51] by increasing oxidative stress, promoting endothelial dysfunction, and enhancing inflammation.

Recent studies have shown an independent positive association between UA and cardiovascular mortality.[52,53] However, there is still conflicting evidence for the results. For instance, numerous epidemiological studies, including prospective, retrospective, cross sectional, and meta-analysis, have not shown an independent association between UA and CVD.[54,55] In contrast, an 8-year follow-up study of 90,393 Taiwanese indicated that hyperuricemia was an independent risk factor of cardiovascular death.[56] Moreover, the Mendelian Randomization Study reported that an increased UA concentration is associated with sudden cardiac death (HR: 2.41; 95% CI, 1.16–5.0) independent of traditional factors.[57] These results suggest that high UA is causally related to adverse cardiovascular outcomes, especially sudden cardiac death. Positive associations have also been demonstrated among specific populations that are at a high risk for CVD, such as those with prevalent type 2 diabetes,[58] hypertension,[59] or a history of CAD.[60]

4 Biomarkers of plaque instability/rupture

4.1 Pregnancy-associated plasma protein-A (PAPP-A)

PAPP-A is a zinc-binding matrix metalloproteinase that belongs to the metzincin superfamily of metalloproteinases. Originally identified in pregnant women, it is produced in the placenta.[61] PAPP-A induces the activation of insulin-derived growth factor-1 (IGF-1), which in turn induces inflammation and lipid uptake that can contribute to atherogenesis and plaque instability.[62]

Two initial studies observed that the PAPP-A concentration is associated with recurrent ischemic events in patients with suspected ACS, independent of TnI.[63, 64] Subsequently, some clinical studies have shown that elevated levels of PAPP-A in patients with stable and unstable CAD are associated with a higher risk of cardiovascular events.[65, 66] In a prospective study, Bonaca, *et al.*[67] found a significant relationship between PAPP-A and cardiovascular death or recurrent ischemic events in 3782 patients with ACS in con-

junction with a contemporary sensitive assay for cTnI. Therefore, circulating PAPP-A is a promising biomarker for risk stratification of ACS. Recently, a 3-vessel virtual histology (VH)-intravascular ultrasound (IVUS) study demonstrated, for the first time, that higher PAPP-A levels are associated with higher 3-vessel thin-cap fibroatheroma burden in patients with CAD.[68] Therefore, PAPP-A might be a useful serum biomarker to predict increased coronary thin-cap fibroatheroma burden and plaque instability.

4.2 Myeloperoxidase (MPO)

MPO, a member of the heme peroxidase family, is produced by polymorphonuclear leukocytes, neutrophils, and monocytes and released in inflammatory conditions. MPO is expressed by macrophages capable of activating MMP and inhibiting TIMP, and it induces low density lipoprotein (LDL) oxidation through hypochlorous acid generation, induces oxidation of ApoA-I, and reduces cholesterol efflux capacity.[69] MPO is considered to be a major contributor in the formation and rupture of plaque. Yunoki, *et al.*[70] observed that MPO levels have a significant inverse correlation with levels of paraoxonase-1 bound to high density lipoprotein (HDL), especially in patients with stable and unstable angina pectoris, which suggests that a mismatch between pro-oxidants and anti-oxidants may contribute to the progression of coronary plaque instability.

An association between MPO levels and CAD risk was first reported in 2001.[71] In another perspective study, Meuwese, *et al.*[72] examined the association of MPO with the risk of CAD development in an initially healthy population in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study. Subsequent prospective and cross-sectional studies addressed the role of MPO as a circulating inflammatory marker in ACS,[73] HF,[74] and CAD.[75] In the CAPTURE trial, Baldus, *et al.*[76] investigated the prognostic information of circulating MPO concentrations in 1090 patients with ACS and investigated the risk stratifications of these patients by multimarker strategies. In contrast, Nicholls, *et al.*[77] demonstrated that MPO concentrations were predictive of cardiovascular events up to 16 h after chest pain. It seems that, despite the initial process of leukocyte activation and MPO release, it is only possible to employ MPO for risk stratification in the early phase from the onset of chest pain. Recently, a large long-term study (Ludwigshafen Risk and Cardiovascular Health) investigated 3036 participants (median follow-up of 7.75 years) and demonstrated that MPO concentrations but not genetic variants at the MPO locus were independently associated with risk for total and cardiovascular mortality in CAD. Collectively, current findings do not provide evidence for a direct causality of MPO in the risk of adverse clinical outcomes, thus the role of MPO in identifying patients at risk for MI is limited. Studies specifically investigating the actual role of MPO are still needed, and routine measurement of this biomarker is not recommended in any clinical settings.

4.3 Matrix metalloproteinases (MMPs)

MMPs are a family of endopeptidases that are secreted by various inflammatory and tumor cells as zymogens and are subsequently activated by proteinases. MMPs have counteracting roles in intimal thickening, which stabilizes plaques and also destroys the extracellular matrix, leading to plaque rupture.[78] The MMPs are grouped into interstitial collagenases that degrade fibrillar collagen (MMP-1, -8, -13, and -14), gelatinases that degrade denatured collagen (MMP-2 and -9), stromelysins that have a broader specificity (MMP-3, -7, -10, and -11), and macrophage elastase (MMP-12) that primarily cleaves elastin.[79]

MMP-2, MMP-8, and MMP-9 have been recognized as proteases that contribute to atherosclerotic plaque rupture and clinical events by degenerating structural components of the plaque matrix.[80–82] Their activity is inhibited by a family of antagonists called tissue inhibitor of MMP (TIMPS). Although TIMP-1 and MMP-9 are associated with cardiovascular death, HF, or both, they are not associated with recurrent MI.[83] MMP-2 is also elevated post-MI[84] and is an independent predictor of all-cause mortality in post-ACS.[85] An elevated MMP-2 activity in plaques is associated with a higher rate of subsequent ischemic cerebrovascular events.[86] In contrast to MMP-2, increased MMP-8 levels in the carotid plaque are associated with an unstable plaque phenotype. High MMP-8 levels in the carotid plaque are associated with the occurrence of a systemic cardiovascular outcome during the follow-up.[87] Recently, Goncalves, *et al.*[88] found that the plasma levels of MMP-7 and -12 are elevated in type 2 diabetes mellitus and that the elevated levels are associated with more severe atherosclerosis and an increased incidence of coronary events.

5 Markers of platelet activation

5.1 Lipoprotein-associated phospholipase A2 (Lp-PLA2)

Lp-PLA2 is a member of the phospholipase A2 superfamily and is also known as platelet-activating factor acetylhydrolase. It is mainly produced by monocytes and macrophages. Lp-PLA2 is able to modify the surface of LDL particles in the phospholipid hydrolysis process, which in turn increases their susceptibility to oxidation.[89] After

LDL oxidation, Lp-PLA2 causes the release of lyso-phosphatidylocholine and oxidized fatty acids, which triggers the inflammatory cascade. The accumulation of lyso-phosphatidylcholine and oxidized fatty acids in the sub-intimal space contributes to the development of the plaque lipid core and promotes the transformation of macrophages into foam cells. Lp-PLA2 activity seems to be essential for its contribution to vulnerable plaques and the occurrence of ACS.

The West of Scotland Coronary Prevention Study was the first study demonstrating an association between elevated Lp-PLA2 levels and cardiovascular events.[90] Subsequent studies demonstrated that Lp-PLA2 activity was an independent predictor of CAD and stroke beyond traditional risk factors in the general population. In 2012, both the American and European guidelines recommended the incorporation of Lp-PLA2 measurements into patients' cardiovascular risk assessment.[91] Although elevated Lp-PLA2 levels have been shown to be associated with an increased cardiovascular risk independent of other covariates, the overall incremental clinical utility of this biomarker remains unclear. Furthermore, two recent large-scale randomized trials failed to show any clinical benefit in stable or unstable CAD patients with the use of an Lp-PLA2 inhibitor.[92,93] These results cast doubt about the potential utility of this biomarker in cardiovascular risk prediction. Thus, further studies are needed to establish the causal role of Lp-PLA2 in cardiovascular events.

5.2 Secretory phospholipase A2 (sPLA2)

The sPLA2 family consists of 10 disulfide-rich isoenzymes of low molecular mass, which is the largest group of this family of enzymes. They are sPLA2-IB, -IIA, -IIC, -IID, -IIE, -IIF, -III, -V, -X, and -XIIA, and these isoenzymes are involved in a variety of biological processes.[94] Out of the sPLA2s, sPLA2-IIA, sPLA2-V, and sPLA2-X have been identified in atherosclerotic lesions and myocardial regions that have sustained ischemic injury.[95–97] This enzyme may contribute to atherogenesis and inflammation by favoring lipoprotein retention with vascular proteoglycans, inducing platelet activation through the prostanoid pathway activation, and facilitating LDL oxidation.[98, 99]

Observational studies have indicated that higher circulating sPLA2-IIA levels and sPLA2 activity are associated with an increased risk of incident and recurrent cardiovascular events (cardiovascular death, AMI, and stroke).[100–102] However, in patients with ACS, the sPLA2-inhibitor varespladib did not reduce the risk of recurrent cardiovascular events, and it significantly increased the risk of MI,[103] Furthermore, Mendelian randomization and phase III randomized controlled trials were in accordance with the fact that a causal role is unlikely.[104] Thus, the clinical value of measuring sPLA2 levels remains unclear.

5.3 Soluble CD40 ligand (sCD40L)

CD40L belongs to the tumor necrosis factor superfamily and is expressed in various cell types, including immune cells (such as lymphocytes, dendritic cells, neutrophils, and macrophages) and nonimmune cells (such as epithelial cells, vascular smooth muscle cells, and endothelial cells).[105] The interaction of CD40L with its receptor CD40 is of particular importance for immunomodulating properties. The surface-expressed CD40L is subsequently cleaved over a period of minutes to hours, generating a soluble fragment (sCD40L) that is also associated with atherosclerosis and plaque instability. Apart from binding to CD40 and thus leading to its activation, sCD40L can also bind to receptors in the platelet surface, thereby leading to its activation and the further secretion of the soluble form in a complex circle of modulation.[105]

Two large, prospective studies (the CAPTURE trial[106] and the Women's Health Study[107]), reported the prognostic value of sCD40L as a biomarker for detecting subsequent cardiovascular risk both in patients with CAD and otherwise healthy individuals. Recently, the Acute Nondisabling Cerebrovascular Events (CHANCE) trial investigated 3044 consecutive patients and demonstrated that elevated sCD40L levels independently predicted recurrent stroke in patients with minor stroke and transient ischemic attack.[108] However, reports in the literature concerning the diagnostic accuracy of sCD40L in patients with AMI have been controversial, and some investigators have demonstrated that sCD40L is not related to the probability of death, MI, or non-fatal recurrent events.[43,109] Furthermore, Liebetrau, *et al*.[110] observed an acute reduction of sCD40L in the setting of early AMI, and they speculated that a reduction in acute platelet activation might be the reason. Further studies are needed in order to specify a definite role for sCD40L in the routine evaluation of patients with suggestive cardiovascular ischemic symptoms.

6 Biomarkers of neurohormonal activation

6.1 Copeptin

Copeptin, a glycosylated 39-amino-acid peptide, is a C-terminal part of the precursor pre-provasopressin (pre-proAVP) and is released in the same amount as AVP. Copeptin is stable and has a half-life of days in plasma, as compared to 5–20 min for AVP.[111] Therefore, copeptin has

been established as a liable biomarker for heart diseases as well as a predictor of mortality in place of AVP. Copeptin is thought to be a novel hallmark of the activation of the hypothalamus-pituitary-adrenals axis.[112] As such, copeptin has received major focus in clinical practice as a marker of cardiovascular events (i.e., AHF, AMI,[113] and stroke[114]) and extra-cardiac conditions (i.e., sepsis[115] and infection[116]).

Recently, Tasevska, *et al.*[117] observed that copeptin could predict CAD development and cardiovascular mortality both in diabetics and non-diabetics. Subjects belonging to the top versus the bottom quartile of copeptin had a > 70% increased risk of dying from CAD. Furthermore, Boeckel, *et al.*[118] found a significant increase of copeptin in patients suffering an AMI but not a direct cardiac release into the coronary circulation in AMI. Therefore, whether the heart also contributes to a release of copeptin into the blood is still a matter of debate.

6.2 Mid-regional-pro-adrenomedullin (MR-proADM)

Adrenomedullin (ADM), a 52-amino acid ringed peptide with C-terminal amidation, was first found in pheochromocytoma cells in the adrenal medulla.[119] ADM is a potent vasodilator synthesized in the adrenal medulla, vascular endothelial cells, the heart, and elsewhere in response to physical stretch and specific cytokines. ADM levels in the heart will elevate as a result of pressure and volume overload. It is difficult to measure plasma ADM levels due to its short half-life and the existence of binding proteins. This peptide can be indirectly quantified by measuring MR-proADM, which is more stable and is manufactured in a one-to-one ratio with active ADM.[120]

Klip, *et al.*[121] demonstrated that MR-proADM is a promising biomarker and has strong prognostic value for mortality and morbidity in patients with HF after an AMI and is superior to NT-proBNP in risk prediction. Bahrmann, *et al.*[122] prospectively investigated the prognostic performance of different biomarkers in unselected older patients (aged 81 ± 6 years) in the emergency department, and found that MR-proADM was the only predictor of cardiovascular deaths. Additionally, MR-proADM is positively associated with brachial pulse pressure and carotid intima-media thickness.[123] Thus, MR-proADM seems to be a promising prognostic biomarker for early atherosclerotic plaque development and subclinical CAD. Furthermore, elevated MR-proADM plasma concentrations are strongly associated with classical cardiovascular risk factors and CAD.[124] Haaf, *et al.*[125] indicated that although MR-proADM did not have any clinical utility in early AMI diagnosis, it provided prognostic value for all-cause mortality. While it is promising for predicting short-term prognosis, more data is necessary before MR-proADM is to be considered ready for prime-time clinical use.

7 Biomarkers of myocardial dysfunction or stress

7.1 Natriuretic peptides

The natriuretic peptides are a closely-related family of ring-shaped peptides involved in sodium and water balance. A number of structurally similar natriuretic peptides have been identified: the atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and Dendroaspis natriuretic peptide (DNP).[126] Of these, ANP and BNP are transcribed and primarily produced in the myocytes of the atria and the ventricles, respectively. In conditions of myocardial stretch, the induction of the BNP gene results in the production and secretion of prohormone, which is cleaved into the biologically more stable N-terminal pro–B-type natriuretic peptide (NT-proBNP).[127]

The ARIC study demonstrated that NT-proBNP is independently associated with incident HF and improves HF risk prediction beyond the traditional risk factors, even among individuals with obesity.[128] Furthermore, in the Multi-Ethnic Study of Atherosclerosis (MESA) of 5592 participants, the authors observed that among asymptomatic individuals of multiple ethnicities, NT-proBNP is an independent predictor of incident CAD and CVD beyond clinical risk factors. A change in NT-proBNP may provide additional prognostic information.[129] The Mid-Regional pro-Atrial Natriuretic Peptide (MR-proANP) is a fragment of the A-type natriuretic peptide prohormone that is produced by cardiomyocytes in response to pressure or fluid overload.[130] The highest plasma concentrations were found in the aorta and pulmonary artery, which is consistent with cardiac production and reflects atrial pressure or transmural stress. MR-proANP is a substantially more stable peptide as compared to N-ANP and ANP due to the assay epitopes being located internally on the proANP molecule.[131] Much like NT-proBNP, MR-proANP is prognostic for an adverse outcome in patients with acutely decompensated HF. In the PRIDE study, Kaplan–Meier curves also showed that MR-proANP was independently prognostic to death out to 4 years of follow up, individually or in a multimarker strategy.[132] Karakas, *et al.*[133] recently observed that MR-proANP was independently associated with recurrent cardiovascular events after adjustment for established risk factors. When both NT-proBNP and MR-proANP were assessed, the results indicated that MR-proANP failed to provide additional prognostic information to NT-proBNP in the population studied. In the current European guidelines, the peptides are

regarded as equal for the diagnosis of both chronic heart failure (CHF) and AHF.[134]

7.2 ST2

ST2 is a member of the interleukin-1 receptor family and exists in two different forms: a transmembrane receptor (ST2L) and a soluble decoy receptor (ST2).[135] Its downstream effects include activation of T-helper type 2 (Th2) cells and production of Th2-associated cytokines.[136]

Studies in patients with AMI,[137,138] AHF,[139] and CHF[140] have reported associations between higher plasma sST2 concentrations and increased risk for mortality and nonfatal adverse cardiac events, such as worsening HF, recurrent MI, and stroke. Dieplinger, *et al.*[141] demonstrated that for stable CAD, increased sST2 was also an independent predictor of long-term all-cause mortality and provided complementary prognostic information to hs-cTnT and NT-proBNP. The Dallas Heart Study investigated a low-risk population, and it was found that sST2 is associated with increased all-cause and cardiovascular mortality.[142] However, it remains unclear what the appropriate ST2 upper reference limit for predicting risk in patients with suspected or proved ACS would be. Data from MERLIN-TIMI 36 suggest that the conventional value of 35 ng/mL might be acceptable, but it is not conclusively known whether gender-based thresholds should be considered.[143] The recommended cut-off for sST2 in CHF is 35 ng/mL.[144] The studies[137,138,141,143,145–148] about cardiovascular risk stratification are summarized in Table 3.

7.3 Endothelin-1 (ET-1)

ET1, a 21-amino acid peptide, is a potent vasoconstrictor and pro-fibrotic hormone that is secreted by vascular endothelial cells, with levels that correlate to shear stress and pulmonary artery pressure.[149]

Elevated ET-1 is associated with short-term in-hospital clinical outcomes and 180-day mortality in hospitalized patients with AHF.[149] ET-1 provided additional prognostic information that was incremental to that yielded by NT-proBNP.[150] However, because of plasma instability, the clinical use of the neurohormone is limited. The C-terminal portion of pro-Endothelin-1 (CTproET1) is the more stable form of ET1 and indirectly measures the activity of the endothelial system. Both in stable CAD and AMI patients, CT-proET-1 has been shown to be associated with cardiovascular death and HF independent of clinical variables, and displayed prognostic value comparable to BNP or NT-proBNP.[151,152]

7.4 Galectin-3 (Gal3)

Gal3 is a glycoprotein-binding, 26 kDa lectin family protein that is secreted by activated cardiac macrophages. It has a pivotal role in atherogenesis through its enhancement of phagocytosis, and it displays a reversal of the inducible-nitric oxide synthase to arginase switch within plaques.[153]

Recently, Maiolino, *et al.*[154] reported that plasma Gal3 can predict cardiovascular death in high-risk patients referred for coronary angiography. Furthermore, Lisowska, *et al.*[155] observed that Gal-3 was an independent risk factor of CAD occurrence, and a Gal-3 concentration > 8.7 ng/mL was an independent predictive indicator of increased risk of all-cause mortality in MI patients during mid-term follow up. Gal-3 may also have functions that are related to the inflammatory cascade following cardiac injury and pathways regulating cardiac contractility.[156] Prior studies revealed that galectin-3 expression is up-regulated in HF and it may be used as a biomarker for the diagnosis and prognosis of HF,[157,158] Furthermore, Gal-3 is a useful biomarker for the diagnosis of HF in patients with preserved ejection

Table 3. Studies using ST-2 for cardiovascular risk stratification.

Studies	*n*	Endpoint	Thresholds	Hazard ratio
ACS[138]	373	All-cause mortality	5–538 pg/mL, 539–3618 pg/mL	2.1,2.2
NSTE-ACS[145]	4432	Cardiovascular death, HF, MI, recurrent ischemia	< 35 ng/mL, ≥ 35 ng/mL	2.08,1.19
MERLIN-TIMI 36 Trial[143]	6560	Cardiovascular death, HF	> 35 μg/L	1.9
STEMI[137]	677	All-cause mortality at 30 days and 1 year	Median	9.34,3.15
LURIC study[141]	1345	All-cause mortality	> 24.6 ng/mL	1.39
AHF[146]	107	All-cause mortality	> 65 ng/mL	1.09
AHF[147]	5306	All-cause mortality	Median	10.3
CHF[148]	876	All-cause and cardiovascular mortality	Quartile (< 30.9 ng/mL, 31–38.3 ng/mL, 38.4–51.1 ng/mL, > 51.1 ng/mL)	1.45,1.55

ACS: acute coronary syndrome; AHF: acute heart failure; CHF: chronic heart failure; HF: heart failure; NSTE-ACS: non-ST-segment-elevation-acute coronary syndrome; STEMI: ST-segment–elevation myocardial infarction.

fraction.[159] Elevated Gal-3 levels are associated with mortality in both AHF and CHF. The diagnostic odds ratio of Gal-3 in predicting mortality in CHF patients was 2.36 (95% CI: 1.71–3.26) and 2.30 (95% CI: 1.76–3.01) in AHF patients.[160] Additionally, Gal-3 was approved by the US Food and Drug Administration (FDA) in 2010 as a new biomarker in the risk stratification of HF. However, current evidence does not support the sole use of Gal-3 for the prognosis evaluation of HF.

7.5 Neuregulin-1 (NRG-1)

NRG-1 is a paracrine growth factor that is released from endothelial cells and binds to a family of ErbB receptors on nearby cardiac myocytes to promote cell survival, growth, and maintenance.[161] To date, more than 15 different protein products encoded by the NRG-1 gene have been described. NRG-1 beta, is the most abundant NRG-1 protein in the cardiac system. NRG-1 ligand exerts its effect in a paracrine manner via the family of ErbB (ErbB2, ErbB3, ErbB4) tyrosine kinase receptors. Various cardiovascular stimuli, such as oxidative stress, ischemia, and exercise, activate the expression of NRG-1.[162] Thus, NRG-1/ErbB4 paracrine signaling in the heart and suggest that this system is involved in cardiac adaptation to various forms of physiologic stress.

Higher NRG-1 levels correlated with more advanced stages of HF and portended a worse prognosis in HF patients with CAD.[163] In an observational cohort of patients with stable CAD referred for PCI, circulating NRG-1 correlated inversely with severity of CAD, while is higher in patients with stress tests that were positive for ischemia.[164] Elevated serum levels of NRG have also been correlated with poor outcomes in patients with HF. Similarly with NT-proBNP, elevated serum levels of NRG may be an inadequate physiologic response to cardiovascular damage, and exogenous administration of NRG may improve cardiovascular function. These findings are consistent with the concept that myocardial NRG-1 is activated in response to ischemia. The potential of NRG-1 as a valuable biomarker of CVD warrants further studies.

8 Biomarkers of microRNAs (miRNAs)

There has been much recent interest in the role of miRNAs in the pathophysiology of cardiovascular disease. MiRNAs are short (-22 nucleotides), noncoding RNA molecules. They exert their function via the seed region, a sequence of six to eight nucleotides that binds to messenger ribonucleic acid (mRNA), the so-called miRNA targets.[165] They typically down regulate translation at the post-expression level and can prevent gene expression through two major pathways: translational repression and mRNA degradation. Real-time quantitative polymerase chain reaction has been the cornerstone for miRNA quantification and remains the most reliable technique for quantitative comparison of miRNA expression levels. Numerous pathways that are affected by miRNA regulation, such as lipid metabolism, glucose homeostasis, vascular integrity and endothelial cell function, are highly involved in CVD.

MiRNAs are present, stable, and detectable in the circulation. Several cardiac miRNAs are detectable in blood early after MI, potentially reducing time to diagnosis.[166] Karakas, *et al*.[167] found a surprisingly strong correlation of single miRNAs with the risk of cardiovascular death and showed their prognostic value in second prevention. Bye, *et al*.[168] and Zampetaki, *et al*.[169] found the combined usefulness of a miRNA panel improved the predictive power of traditional Framingham risk models, but no single miRNA conferred a clinically significant change in risk of acute MI. More recently, miRNAs can act as a novel biomarker for platelet reactivity and can be affected by the administration of antiplatelet therapy. Their platelet origin could make circulating miRNAs particularly relevant in the context of CVD. However, current miRNA detection techniques are time consuming and do not allow for the rapid diagnosis required in patients with MI and their clinical benefits beside current diagnostic tools remain unclear.

9 Future perspectives

There are large numbers of emerging novel biomarkers; however, the roles and biochemistry of these markers as they relate to the risk of future cardiovascular events in individuals with and without CAD and their clear clinical utility have not yet been fully elucidated. Accordingly, it is difficult to draw specific conclusions from the current evidence regarding the mechanisms through which a biomarker could affect the prognosis. Although there is evidence that combining biomarkers may increase the accuracy of certain tests, the optimal combinations for diagnosis or prognosis need to be defined. GDF-15 and ST2 provide cardiovascular risk stratification information in patients with stable CAD or ACS. An evaluation of hsCRP, fibrinogen, MPO may be considered to related to CVD and provide additional information beyond traditional risk factors for cardiovascular risk, respectively. Additionally, both NT-proBNP and MR-proANP are regarded as equal for diagnosing HF according to the European guideline. Though biomarkers have significantly improved our delivery of care, it is important to remember that all biomarkers work in conjunction with other clinical information including history, physical, and

other laboratory and radiographic findings. They are aids for diagnosis and management and must be interpreted within their clinical context and not solely acted upon. It is also important to note that due to the multi-factorial pathogenesis of CAD, detailed risk stratification remains a complex process. Further research is needed to identify new biomarkers and to determine if a multi-marker strategy of established and novel biomarkers is a feasible approach for better risk stratification. The application of powerful novel discovery platforms, such as genomics, proteomics, metabolomics and lipidomics are still in the developmental stages, but there is potential for rapid growth. Translating these discoveries into clinical practice will be critical for reducing the population burden of CVD.

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