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 protein all 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 farther 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. Cardiac troponin I (cTnI) and T (cTnT) are proteins that are unique to the heart and are specific and sensitive biomarkers of myocardial damage. 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. 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. 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.

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. 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. 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.

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). 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. Kabekkodu, et al. 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.

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.\(^{[20-22]}\) HS-CRP that detects lower levels of CRP (< 5 mg/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-inhibitory cytokine-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 (NSTEMI) and for 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
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: \( \alpha \), \( \beta \), and \( \gamma \) and 8–15% of circulating fibrinogen in healthy individuals contains a \( \gamma \) chain (\( \gamma A/\gamma^\prime \)). Recently, a large prospective study showed a positive association of \( \gamma^\prime \) fibrinogen with incident CAD, ischemic stroke, peripheral artery disease, HF, and cardiovascular deaths.\(^{48}\) This observation has suggested that \( \gamma A/\gamma^\prime \) fibrinogen is a causal risk factor for CVD. Both \textit{in vitro} and \textit{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, \textit{et al.}\(^{67}\) found a significant relationship between PAPP-A and cardiovascular death or recurrent ischemic events in 3782 patients with ACS in con-

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

| Study population   | n   | Endpoint                                                                 | Thresholds                                      | Hazard ratio |
|--------------------|-----|--------------------------------------------------------------------------|------------------------------------------------|--------------|
| ALPS-AMF\(^{38}\)  | 430 | All-cause death, MI, stroke, or hospitalization due to congestive HF    | \(< 1221 \text{ng/L}, \geq 1221 \text{ng/L}\)  | 1.001        |
| PLATO trial\(^{39}\) | 16,876 | Cardiovascular death, spontaneous MI, and stroke | Quartile \(< 1145 \text{ng/L}, 1145–1550 \text{ng/L}, 1550–2219 \text{ng/L}, > 2219 \text{ng/L}\) | 1.4          |
| IABP-SHOCK II\(^{40}\) | 600 | All-cause mortality                                                     | Median                                         | 1.88         |
| Suspected AMF\(^{41}\) | 1247 | All-cause death, AMI                                                     | \(< 1200 \text{ng/L}, 1200–1800 \text{ng/L}, > 1800 \text{ng/L}\) | 19.2, 20.1   |
| NSTE-ACS\(^{42}\)   | 1146 | Deaths and nonfatal MI                                                   | Median                                         | 2.4          |
| AtheroGene\(^{43}\)  | 1781 | Nonfatal MI, cardiovascular mortality                                    | \(\geq 1499 \text{ng/L}\)                      | 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.
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. MPO is considered to be a major contributor in the formation and rupture of plaque. Yunoki, et al. observed that MPO levels have a significant inverse correlation 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. In another prospective study, Meuwese, et al. 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, HF, and CAD. In the CAPTURE trial, Baldus, et al. 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. 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. 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.

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. 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. MMP-2 is also elevated post-MI and is an independent predictor of all-cause mortality in post-ACS. An elevated MMP-2 activity in plaques is associated with a higher rate of subsequent ischemic cerebrovascular events. 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. Recently, Goncalves, et al. 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.
LDL oxidation, Lp-PLA2 causes the release of lyso-phosphatidylcholine 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, -IV, -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.\textsuperscript{[112]} As such, copeptin has received major focus in clinical practice as a marker of cardiovascular events (i.e., AHF, AMI\textsuperscript{[113]} and stroke\textsuperscript{[114]}) and extra-cardiac conditions (i.e., sepsis\textsuperscript{[115]} and infection\textsuperscript{[116]}).

Recently, Tasevska, et al.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[120]}

Klip, et al.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[124]} Haaf, et al.\textsuperscript{[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).\textsuperscript{[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).\textsuperscript{[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.\textsuperscript{[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. 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.\textsuperscript{[129]} 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.\textsuperscript{[130]} 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.\textsuperscript{[131]} Karakas, et al.\textsuperscript{[132]} 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). 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 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]

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

| Studies | n   | Endpoint                                   | Thresholds                | Hazard ratio |
|---------|-----|--------------------------------------------|---------------------------|--------------|
| ACS     | 373 | All-cause mortality                         | 5–538 pg/mL,             | 2.1,2.2      |
|         |     |                                            | 539–3618 pg/mL           |              |
| NSTE-ACS|4432 | Cardiovascular death, HF, MI, recurrent ischemia | < 35 ng/mL, ≥ 35 ng/mL | 2.08,1.19    |
| MERLIN-TIMI 36 Trial[141] | 6560 | Cardiovascular death, HF | > 35 μg/L | 1.9          |
| STEMI[142] | 677  | All-cause mortality at 30 days and 1 year | Median                  | 9.34,3.15    |
| LURIC study[143] | 1345 | All-cause mortality                        | > 24.6 ng/mL            | 1.39         |
| AHF[144] | 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    |

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 arginine 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 fraction. [159]
fraction. 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. 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. 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. 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. In an observational cohort of patients with stable CAD referred for PCI, circulating NRG-1 correlated inversely with severity of CAD, while higher in patients with stress tests that were positive for ischemia. 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. 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. Karakas, et al. 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. and Zampetaki, et al. 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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References

1. Forouzanfar MH, Alexander L, Anderson HR, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990–2013: a systematic analysis for the global burden of disease study 2013. Lancet 2015; 386: 2287–2323.

2. Hozawa A, Folsom AR, Sharrett AR, et al. Absolute and attributable risks of cardiovascular disease incidence in relation to optimal and borderline risk factors: comparison of African American with white subjects—atherosclerosis risk in communities study. Arch Intern Med 2007; 167: 573–579.

3. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001; 69: 89–95.

4. Redberg RF, Vogel RA, Criqui MH, et al. 34th Bethesda conference: task force #3—what is the spectrum of current and emerging techniques for the noninvasive measurement of atherosclerosis? J Am Coll Cardiol 2003; 41: 1886–1898.

5. Hoff J, Wehner W, Nambi V. Troponin in cardiovascular disease prevention: updates and future direction. Curr Atheroscler Rep 2016; 18: 12.

6. Safford MM, Parmar G, Barasch CS, et al. Hospital laboratory reporting may be a barrier to detection of ‘microrise’ myocardial infarction in the US: an observational study. BMC Health Serv Res 2013; 13: 162.

7. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Eur Heart J 2012; 33: 2551–2567.

8. Korley FK, Jaffe AS. Preparing the United States for high-sensitivity cardiac troponin assays. J Am Coll Cardiol 2013; 61: 1753–1758.

9. Roffi M, Patrono C, Collet JP, et al. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2016; 37: 267–315.

10. Cullen L, Mueller C, Parsonage WA, et al. Validation of high-sensitivity troponin I in a 2-hour diagnostic strategy to assess 30-day outcomes in emergency department patients with possible acute coronary syndrome. J Am Coll Cardiol 2013; 62: 1242–1249.

11. Haaf P, Drexler B, Reichlin T, et al. High-sensitivity cardiac troponin in the distinction of acute myocardial infarction from acute cardiac noncoronary artery disease. Circulation 2012; 126: 31–40.

12. Everett BM, Brooks MM, Vlachos HE, et al. Troponin and cardiac events in stable ischemic heart disease and diabetes. N Engl J Med 2015; 373: 610–620.

13. Pelsers MM, Hermens WT, Glazt JF. Fatty acid-binding proteins as plasma markers of tissue injury. Clin Chim Acta 2005; 352: 15–35.

14. McMahon CG, Lamont JV, Curtin E, et al. Diagnostic accuracy of heart-type fatty acid-binding protein for the early diagnosis of acute myocardial infarction. Tam J Emerg Med 2012; 30: 267–274.

15. Gami BN, Patel DS, Haridas N, et al. Utility of heart-type fatty acid binding protein as a new biochemical marker for the early diagnosis of acute coronary syndrome. J Clin Diagn Res 2015; 9: Bc22–Bc24.

16. Kabekkod SP, Mananje SR, Saya RP. A study on the role of heart type fatty acid binding protein in the diagnosis of acute myocardial infarction. J Clin Diagn Res 2016; 10: Oc07–Oc10.

17. Otuki Y, Watanabe T, Takahashi H, et al. Association of heart-type fatty acid-binding protein with cardiovascular risk factors and all-cause mortality in the general population: the Takahata study. PLoS One 2014; 9: e94834.

18. Bassuks S, Rifai N, Ridker PM. High-sensitivity C-reactive protein: clinical importance. Curr Probl Cardiol 2004; 29: 439–493.

19. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. Circulation 2003; 107: 398–404.

20. Ridker PM, Hennekens CH, Buring JE, et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000; 342: 836–843.

21. Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997; 336: 973–979.

22. Jaber BL, Madias NE. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005; 352: 1603–1605.

23. Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive
protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 2010; 375: 132–140.

24 Oemrawsingh RM, Cheng JM, Akkerhuis KM, *et al.* High-sensitivity C-reactive protein predicts 10-year cardiovascular outcome after percutaneous coronary intervention. *EuroIntervention* 2016; 12: 345–351.

25 Vlachopoulos C, Xaplanteris P, Aboyans V, *et al.* The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology working group on peripheral circulation: endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society. *Atherosclerosis* 2015; 241: 507–532.

26 Lane T, Wassef N, Poole S, *et al.* Infusion of pharmaceuti-
cal-grade natural human C-reactive protein is not proin-
flammatory in healthy adult human volunteers. *Circ Res* 2014; 114: 672–676.

27 Noveck R, Stroes ES, Flaim JD, *et al.* Effects of an antisense oligonucleotide inhibitor of C-reactive protein synthesis on the endotoxin challenge response in healthy human male volunteers. *J Am Heart Assoc* 2014; 3.

28 Elliott P, Chambers JC, Zhang W, *et al.* Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA* 2009; 302: 37–48.

29 Wiklund FE, Bennet AM, Magnusson PK, *et al.* Macrophage inhibitory cytokine-1 (mic-1/gdf15): a new marker of all-cause mortality. *Aging Cell* 2010; 9: 1057–1064.

30 Wallentin L, Hijazi Z, Andersson U, *et al.* Growth differentia-
tion factor 15, a marker of oxidative stress and inflamma-
tion, for risk assessment in patients with atrial fibrillation: insights from the apixaban for reduction in stroke and other thromboembolic events in atrial fibrillation (aristotle) trial. *Circulation* 2014; 130: 1847–1858.

31 Wollert KC, Kempf T. Growth differentiation factor 15 in heart failure: an update. *Curr Heart Fail Rep* 2012; 9: 337–345.

32 Kempf T, Zarbock A, Widera C, *et al.* Gdf-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat Med* 2011; 17: 581–588.

33 Daniels LB, Clpton P, Laughlin GA, *et al.* Growth-differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the Rancho Bernardo Study. *Circulation* 2011; 123: 2101–2110.

34 Eggers KM, Kempf T, Larson A, *et al.* Evaluation of temporal changes in cardiovascular biomarker concentrations improves risk prediction in an elderly population from the community. *Clin Chem* 2016; 62: 485–493.

35 Cotter G, Voors AA, Prescott MF, *et al.* Growth differentia-
tion factor 15 (gdf-15) in patients admitted for acute heart failure: results from the relax-ahf study. *Eur J Heart Fail* 2015; 17: 1133–1143.

36 Wollert KC, Kempf T, Lagerqvist B, *et al.* Growth differentia-
tion factor 15 for risk stratification and selection of an in-
vasive treatment strategy in non ST-elevation acute coronary syndrome. *Circulation* 2007; 116: 1540–1548.

37 Ho JE, Mahajan A, Chen MH, *et al.* Clinical and genetic correlates of growth differentiation factor 15 in the community. *Clin Chem* 2012; 58: 1582–1591.

38 Minamisawa M, Motoki H, Izawa A, *et al.* Comparison of inflammatory biomarkers in outpatients with prior myocardial infarction. *Int Heart J* 2016; 57: 11–17.

39 Hagstrom E, James SK, Bertilsson M, *et al.* Growth differ-
entiation factor-15 level predicts major bleeding and cardio-
vascular events in patients with acute coronary syndromes: results from the PLATO study. *Eur J Heart J* 2016; 37: 1325–1333.

40 Fuernau G, Poenisch C, Eitel I, *et al.* Growth-differentiation factor 15 and osteoprotegerin in acute myocardial infarction complicated by cardiogenic shock: a biomarker substudy of the labp-shock II-trial. *Eur J Heart Fail* 2014; 16: 880–887.

41 Damman P, Kempf T, Windhausen F, *et al.* Growth-differ-
tentiation factor 15 for long-term prognostication in patients with non-ST-elevation acute coronary syndrome: an Invasive versus Conservative Treatment in Unstable coronary Syndromes (ICTUS) substudy. *Int J Cardiol* 2014; 172: 356–363.

42 Widera C, Pencina MJ, Bobadilla M, *et al.* Incremental prognostic value of biomarkers beyond the Grace (Global Registry of Acute Coronary Events) score and high-sensitivity cardiac troponin I in non-ST-elevation acute coronary syndrome. *Clin Chem* 2013; 59: 1497–1505.

43 Schnabel RB, Yin X, Lanson MG, *et al.* Multiple inflammato-
ry biomarkers in relation to cardiovascular events and mortality in the community. *Arterioscler Thromb Vasc Biol* 2013; 33: 1728–1733.

44 Wollert KC, Kempf T, Peter T, *et al.* Prognostic value of growth-differentiation factor-15 in patients with non-ST-eleva-
tion acute coronary syndrome. *Circulation* 2007; 115: 962–971.

45 Lowe GD. Fibrinogen assays for cardiovascular risk as-
essment. *Clin Chem* 2010; 56: 693–695.

46 Danesh J, Lewington S, Thompson SG, *et al.* Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-
analysis. *JAMA* 2007; 297: 2235–2241.

47 Kaptoge S, Di Angelantonio E, Pennells L, *et al.* High-sensitivity cardiac troponin T in non-ST-elevation acute coronary *Int J Cardiol* 2017; 241: 588.

48 Danesh J, Lewington S, Thompson SG, *et al.* Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-
analysis. *JAMA* 2007; 297: 2235–2241.

49 Van Dijk MJ, van der Meer RM, *et al.* Association of fibrinogen with major cardiovascular disease: The Atherosclerosis Risk in Communities (ARIC) study. *Arterioscler Thromb Vasc Biol* 2015; 35: 2700–2706.

50 Becatti M, Marcucci R, Bruschi G, *et al.* Oxidative modifi-
cation of fibrinogen is associated with altered function and structure in the subacute phase of myocardial infarction. *Arterioscler Thromb Vasc Biol* 2014; 34: 1355–1361.

51 Fabbri E, Sarafini M, Colic Baric I, *et al.* Effect of plasma uric acid on antioxidant capacity, oxidative stress, and insulin sensitivity in obese subjects. *Diabetes* 2014; 63: 976–981.

52 Jin YL, Zhu T, Xu L, *et al.* Uric acid levels, even in the normal range, are associated with increased cardiovascular risk: the Guangzhou Biobank Cohort Study. *Int J Cardiol* 2013; 168: 2238–2241.
protein a: a biomarker in acute ST-elevation myocardial infarction (STEMI). *Ann Med* 2006; 38: 221−228.

67. Bonaca MP, Scirica BM, Sabatine MS, et al. Prospective evaluation of pregnancy-associated plasma protein-a and outcomes in patients with acute coronary syndromes. *J Am Coll Cardiol* 2012; 60: 332−338.

68. Wu XF, Yang M, Qu AJ, et al. Level of pregnancy-associated plasma protein-a correlates with coronary thin-cap fibroatheroma burden in patients with coronary artery disease: novel findings from 3-vessel virtual histology intravascular ultrasound assessment. *Medicine* 2016; 95: e2563.

69. Nicholls SJ, Hazen SL. Myeloperoxidase and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2005; 25: 1102−1111.

70. Yunoki K, Naruko T, Inaba M, et al. Gender-specific correlation between plasma myeloperoxidase levels and serum high-density lipoprotein-associated paraoxonase-1 levels in patients with stable and unstable coronary artery disease. *Atherosclerosis* 2013; 231: 308−314.

71. Zhang R, Brennan ML, Fu X, et al. Association between myeloperoxidase levels and risk of coronary artery disease. *JAMA* 2001; 286: 2136−2142.

72. Meuwese MC, Stroes ES, Hazen SL, et al. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the epic-norfolk prospective population study. *J Am Coll Cardiol* 2007; 50: 159−165.

73. Sawicki M, Sypniewska G, Kozinski M, et al. Diagnostic efficacy of myeloperoxidase for the detection of acute coronary syndromes. *Eur J Clin Invest* 2011; 41: 667−671.

74. Tang WH, Tong W, Troughton RW, et al. Prognostic value and echocardiographic determinants of plasma myeloperoxidase levels in chronic heart failure. *J Am Coll Cardiol* 2007; 49: 2364−2370.

75. Cavusoglu E, Ruwende C, Eng C, et al. Usefulness of baseline plasma myeloperoxidase levels as an independent predictor of myocardial infarction at two years in patients presenting with acute coronary syndrome. *Am J Cardiol* 2007; 99: 1364−1368.

76. Baldus S, Heeschen C, Meintz T, et al. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation* 2003; 108: 1440−1445.

77. Nicholls SJ, Tang WH, Brennan D, et al. Risk prediction with serial myeloperoxidase monitoring in patients with acute chest pain. *Clin Chem* 2011; 57: 1762−1770.

78. Newby AC. Metalloproteinases promote plaque rupture and myocardial infarction: a persuasive concept waiting for clinical translation. *Matrix Biol* 2015; 44: 46−157−166.

79. Gu W, Liu W, Yang X, et al. Cutis laxa: analysis of metalloproteinases and extracellular matrix expression by immunohistochemistry and histochemistry. *Eur J Dermatol* 2011; 21: 717−721.

80. Molloy KJ, Thompson MM, Jones JL, et al. Unstable carotid plaques exhibit raised matrix metalloproteinase-8 activity. *Circulation* 2004; 110: 337−343.

81. Kunte H, Amberger N, Busch MA, et al. Markers of insta-
bility in high-risk carotid plaques are reduced by statins. J Vasc Surg 2008; 47: 513–522.
82 Wang LX, Lu SZ, Zhang WJ, et al. Comparison of high sensitivity C-reactive protein and matrix metalloproteinase 9 in patients with unstable angina between with and without significant coronary artery plaques. Chin Med J (Engl) 2011; 124: 1657–1661.
83 Kelly D, Khan SQ, Thompson M, et al. Plasma tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9: novel indicators of left ventricular remodelling and prognosis after acute myocardial infarction. Eur Heart J 2008; 29: 2116–2124.
84 White HD, Held C, Stewart R, et al. Secretory phospholipase a2 group v: lesion distribution, activation by arterial proteoglycans, and induction in aorta by a western diet. Arterioscler Thromb Vasc Biol 2006; 26: 1579–1585.
85 Leitinger N, Watson AD, Hama SY, et al. Role of group ii secretory phospholipase a2 in atherosclerosis: 2. Potential involvement of biochemically active oxidized phospholipids. Arterioscler Thromb Vasc Biol 1999; 19: 1291–1298.
86 Henderson WR, Jr, Chi EY, Bollinger JG, et al. Importance of group X-secreted phospholipase A2 in allergen-induced airway inflammation and remodeling in a mouse asthma model. J Exp Med 2007; 204: 865–877.
87 Mallat Z, Benessiano J, Simon T, et al. Circulating secretory phospholipase a2 activity and risk of incident coronary events in healthy men and women: the epic-norfolk study. Arterioscler Thromb Vasc Biol 2007; 27: 1177–1183.
88 Lind L, Simon T, Johansson L, et al. Circulating levels of secretory- and lipoprotein-associated phospholipase a2 activities: relation to atherosclerotic plaques and future all-cause mortality. Eur Heart J 2012; 33: 2946–2954.
89 Ryu SK, Mallat Z, Benessiano J, et al. Phospholipase A2 enzymes, high-dose atorvastatin, and prediction of ischemic events after acute coronary syndromes. Circulation 2012; 125: 757–766.
90 Nicholls SJ, Kastelein JJ, Schwartz GG, et al. Varespladib and cardiovascular events in patients with an acute coronary syndrome: the VISTA-16 randomized clinical trial. JAMA 2014; 311: 252–262.
91 Nelson MR, Tipney H, Painter JL, et al. The support of human genetic evidence for approved drug indications. Nat Genet 2015; 47: 856–860.
92 Anand SX, Viles-Gonzalez JF, Badimon JJ, et al. Membrane-associated CD40L and sCD40L in atherothrombotic disease. Thromb Haemost 2003; 90: 377–384.
93 Heeschen C, Dimmeler S, Hamm CW, et al. Soluble CD40 ligand in acute coronary syndromes. N Engl J Med 2003; 348: 1104–1111.
94 Schönbeck U, Varo N, Libby P, et al. Soluble CD40L and cardiovascular risk in women. Circulation 2001; 104: 2266–2268.
95 Li J, Wang Y, Lin J, et al. Soluble cd40l is a useful marker to predict future strokes in patients with minor stroke and transient ischemic attack. Stroke 2015; 46: 1990–1992.
96 Plaikner M, Peer A, Falkensammer G, et al. Lack of association of soluble CD40 ligand with the presence of acute myocardial infarction or ischemic stroke in the emergency department. Clin Chem 2009; 55: 175–178.
97 Liebetrau C, Hoffmann J, Dorr O, et al. Release kinetics of inflammatory biomarkers in a clinical model of acute myocardial infarction. Circ Res 2015; 116: 867–875.
98 Bolignano D, Cabassi A, Fiaccadori E, et al. Copeptin...
(CTproAVP), a new tool for understanding the role of vasopressin in pathophysiology. *Clin Chem Lab Med* 2014; 52: 1447–1456.

112 Katan M, Morgenthaler N, Widmer I, et al. Copetin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level. *Neuro Endocrinol Lett* 2008; 29: 341–346.

113 Sorensen NA, Shah AS, Ojeda FM, et al. High-sensitivity troponin and novel biomarkers for the early diagnosis of non-ST-segment elevation myocardial infarction in patients with atrial fibrillation. *Eur Heart J Acute Cardiovasc Care*. 2016; 5: 419–427.

114 Greisenegger S, Segal HC, Burgess AI, et al. Copetin and long-term risk of recurrent vascular events after transient ischemic attack and ischemic stroke: population-based study. *Stroke* 2015; 46: 3117–3123.

115 Lee JH, Chan YH, Lai OF, et al. Vasopressin and copeptin levels in children with sepsis and septic shock. *Intensive Care Med* 2013; 39: 747–753.

116 Alcoba G, Manzano S, Lacroix L, et al. Proadrenomedullin and copeptin in pediatric pneumonia: a prospective diagnostic accuracy study. *BMC Infect Dis* 2015; 15: 347.

117 Tasevska I, Enhorning S, Persson M, et al. Copetin predicts coronary artery disease cardiovascular and total mortality. *Heart* 2016; 102: 127–132.

118 Boeckel JN, Oppermann J, Anadolu R, et al. Analyzing the release of copeptin from the heart in acute myocardial infarction using a transcoronary gradient model. *Sci Rep* 2016; 6: 20812.

119 Klip IT, Voors AA, Anker SD, et al. Prognostic value of mid-regional pro-adrenomedullin in patients with heart failure after an acute myocardial infarction. *Heart* 2011; 97: 892–898.

120 Bahmann P, Christ M, Hofner B, et al. Prognostic value of different biomarkers for cardiovascular death in unselected older patients in the emergency department. *Eur Heart J Acute Cardiovasc Care* 2016; 5: 568–578.

121 Gottsater M, Ford LB, Ostling G, et al. Adrenomedullin is a marker of carotid plaques and intima-media thickness as well as brachial pulse pressure. *J Hypertens* 2013; 31: 1959–1965.

122 Neumann JT, Tzikas S, Funke-Kaiser A, et al. Association of mr-proadrenomedullin with cardiovascular risk factors and subclinical cardiovascular disease. *Atherosclerosis* 2013; 228: 451–459.

123 Haaf P, Twerenbold R, Reichlin T, et al. Mid-regional pro-adrenomedullin in the early evaluation of acute chest pain patients. *Int J Cardiol* 2013; 168: 1048–1055.

124 Cea LB. Natriuretic peptide family: new aspects. *Curr Med Chem Cardiovasc Hematol Agents* 2005; 3: 87–98.

125 Mueller T, Gegenhuber A, Dieplinger B, et al. Long-term stability of endogenous b-type natriuretic peptide (BNP) and amino terminal proBNP (NT-proBNP) in frozen plasma samples. *Clin Chem Lab Med* 2004; 42: 942–944.

126 Ndumele CE, Matsushita K, Sang Y, et al. N-terminal pro-brain natriuretic peptide and heart failure risk among individuals with and without obesity: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 2016; 133: 631–638.

127 Daniels LB, Clopton P, deFilippi CR, et al. Serial measurement of n-terminal pro-b-type natriuretic peptide and cardiac troponin t for cardiovascular disease risk assessment in the Multi-Ethnic Study of Atherosclerosis (MESA). *Am Heart J* 2015; 170: 1170–1183.

128 Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *N Engl J Med* 1998; 339: 321–328.

129 Ala-Kopsala M, Magga J, Peuhkurinen K, et al. Molecular heterogeneity has a major impact on the measurement of circulating n-terminal segments of a- and b-type natriuretic peptides. *Clin Chem* 2004; 50: 1576–1588.

130 Shah RV, Truong QA, Gaggin HK, et al. Mid-regional pro-atrial natriuretic peptide and pro-adrenomedullin testing for the diagnostic and prognostic evaluation of patients with acute dyspnoea. *Eur Heart J* 2012; 33: 2197–2205.

131 Karakas M, Jaensch A, Breetling LP, et al. Prognostic value of midregional pro-a-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide in patients with stable coronary heart disease followed over 8 years. *Clin Chem* 2014; 60: 1441–1449.

132 McMurray JJ, Adamopoulos S, Anker SD, et al. Esc guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: the task force for the diagnosis and treatment of acute and chronic heart failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the esc. *Eur Heart J* 2012; 33: 1787–1847.

133 Januzzi JL, Jr. S2 as a cardiovascular risk biomarker: from the bench to the bedside. *J Cardiovasc Transl Res* 2013; 6: 493–500.

134 Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005; 23: 479–490.

135 Dhillon OS, Narayan HK, Khan SQ, et al. Pre-discharge risk stratification in unselected stemi: is there a role for ST2 or its natural ligand IL-33 when compared with contemporary risk markers? *Int J Cardiol* 2013; 167: 2182–2188.

136 Demyanets S, Speidl WS, Tentzeris I, et al. Soluble ST2 and interleukin-33 levels in coronary artery disease: relation to disease activity and adverse outcome. *PLoS One* 2014; 9: e95055.

137 Manzano-Fernandez S, Mueller T, Pasqual-Figal D, et al. Usefulness of soluble concentrations of interleukin family member ST2 as predictor of mortality in patients with acutely decompensated heart failure relative to left ventricular ejection fraction. *Am J Cardiol*. 2011; 107: 259–267.

138 Ky B, French B, McCloskey K, et al. High-sensitivity ST2

http://www.jgc301.com; jgc@mail.sciencep.com | Journal of Geriatric Cardiology
for prediction of adverse outcomes in chronic heart failure. *Circ Heart Fail* 2011; 4: 180–187.

141 Dieplinger B, Egger M, Haltmayer M, *et al.* Increased soluble ST2 predicts long-term mortality in patients with stable coronary artery disease: results from the ludwigshafen risk and cardiovascular health study. *Clin Chem.* 2014; 60: 530–540.

142 Chen LQ, de Lemos JA, Das SR, *et al.* Soluble ST2 is associated with all-cause and cardiovascular mortality in a population-based cohort: the Dallas heart study. *Clin Chem* 2013; 59: 536–546.

143 Kohli P, Bonaca MP, Kakkar R, *et al.* Role of ST2 in non-st-elevation acute coronary syndrome in the merlin-timi 36 trial. *Clin Chem* 2012; 58: 257–266.

144 Daniels LB, Bayes-Genis A. Using ST2 in cardiovascular patients: a review. *Future Cardiol* 2014; 10: 525–539.

145 O’Malley RG, Bonaca MP, Scirica BM, *et al.* Prognostic performance of multiple biomarkers in patients with non-ST-segment elevation acute coronary syndrome: analysis from the merlin-timi 36 trial (metabolic efficiency with ranolazine for less ischemia in non-ST-elevation acute coronary syndromes-thrombolysis in myocardial infarction 36). *J Am Coll Cardiol* 2014; 63: 1644–1653.

146 Pascual-Figal DA, Manzano-Fernandez S, Boronat M, *et al.* Soluble sST2, high-sensitivity troponin t- and n-terminal pro-b-type natriuretic peptide: complementary role for risk stratification in acutely decompensated heart failure. *Eur J Heart Fail* 2011; 13: 718–725.

147 Lassus J, Jayat E, Mueller C, *et al.* Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: the Multinational Observational Cohort on Acute Heart Failure (MOCA) study. *Int J Cardiol* 2013; 168: 2186–2194.

148 Bayes-Genis A, de Antonio M, Vila J, *et al.* Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: S12 versus galectin-3. *J Am Coll Cardiol* 2014; 63: 158–166.

149 Davenport AP, Hyndman KA, Dhaun N, *et al.* Endothelin. *Pharmacol Rev* 2016; 68: 357–418.

150 Perez AL, Grodin JL, Wu Y, *et al.* Increased mortality with elevated plasma endothelin-1 in acute heart failure: an ascend-hf biomarker substudy. *Eur J Heart Fail* 2016; 18: 290–297.

151 Khan SQ, Dhillon O, Struck J, *et al.* C-terminal pro-endothelin-1 offers additional prognostic information in patients after acute myocardial infarction: Acute Myocardial infarction Peptide (LAMP) study. *Am Heart J* 2007; 154: 736–742.

152 Sabatine MS, Morrow DA, de Lemos JA, *et al.* Evaluation of multiple biomarkers of cardiovascular stress for risk prediction and guiding medical therapy in patients with stable coronary disease. *Circulation* 2012; 125: 233–240.

153 Mackinnon AC, Liu X, Hadoke PW, *et al.* Inhibition of galectin-3 reduces atherosclerosis in apolipoprotein E-deficient mice. *Glycobiology* 2013; 23: 654–663.

154 Maiolino G, Rossitto G, Pedon L, *et al.* Galectin-3 predicts long-term cardiovascular death in high-risk patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2015; 35: 725–732.

155 Lisowska A, Knapp M, Tycinska A, *et al.* Predictive value of galectin-3 for the occurrence of coronary artery disease and progression after myocardial infarction and its association with carotid int values in these patients: a mid-term prospective cohort study. *Atherosclerosis* 2016; 246: 309–317.

156 Dunic J, Dabelic S, Flogel M. Galectin-3: an open-ended story. *Biochim Biophys Acta* 2006; 1760: 616–635.

157 van Kimmenade RR, Januzzi JL, Jr., Ellinor PT, *et al.* Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure. *J Am Coll Cardiol* 2006; 48: 1217–1224.

158 Lok DJ, Van Der Meer P, de la Porte PW, *et al.* Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the deal-hf study. *Clin Res Cardiol* 2010; 99: 322–328.

159 Yin QS, Shi B, Dong L, *et al.* Comparative study of galecin-3 and b-type natriuretic peptide as biomarkers for the diagnosis of heart failure. *J Geriatr Cardiol* 2014; 11: 79–82.

160 Chen YS, Gi WT, Liao TY, *et al.* Using the galectin-3 test to predict mortality in heart failure patients: a systematic review and meta-analysis. *Biomark Med* 2016; 10: 329–342.

161 Lee KF, Simon H, Chen H, *et al.* Requirement for neuregulin receptor erb2b in neural and cardiac development. *Nature* 1995; 378: 394–398.

162 Kuramochi Y, Cote GM, Guo X, *et al.* Cardiac endothelial cells regulate reactive oxygen species-induced cardiomyocyte apoptosis through neuregulin-1beta/erb4 signaling. *J Biol Chem* 2004; 279: 51141–51147.

163 Lemmens K, Doggen K, De Keulenaer GW. Role of neuregulin-1/erb2 signaling in cardiovascular physiology and disease: implications for therapy of heart failure. *Circulation* 2007; 116: 954–960.

164 Geisberg CA, Wang G, Safa RN, *et al.* Circulating neuregulin-1beta levels vary according to the angiographic severity of coronary artery disease and ischemia. *Coron Artery Dis* 2011; 22: 577–582.

165 Barwari T, Joshi A, Mayr M. Circulating miRNA strongly predict cardiovascular death in high-risk patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2015; 35: 725–732.

155 Lisowska A, Knapp M, Tycinska A, *et al.* Predictive value of galectin-3 for the occurrence of coronary artery disease and progression after myocardial infarction and its association with carotid int values in these patients: a mid-term prospective cohort study. *Atherosclerosis* 2016; 246: 309–317.

166 Barwari T, Joshi A, Mayr M. Circulating miRNA strongly predict cardiovascular death in high-risk patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2015; 35: 725–732.

167 Dunic J, Dabelic S, Flogel M. Galectin-3: an open-ended story. *Biochim Biophys Acta* 2006; 1760: 616–635.

168 van Kimmenade RR, Januzzi JL, Jr., Ellinor PT, *et al.* Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure. *J Am Coll Cardiol* 2006; 48: 1217–1224.

169 Yin QS, Shi B, Dong L, *et al.* Comparative study of galecin-3 and b-type natriuretic peptide as biomarkers for the diagnosis of heart failure. *J Geriatr Cardiol* 2014; 11: 79–82.

170 Chen YS, Gi WT, Liao TY, *et al.* Using the galectin-3 test to predict mortality in heart failure patients: a systematic review and meta-analysis. *Biomark Med* 2016; 10: 329–342.

171 Lee KF, Simon H, Chen H, *et al.* Requirement for neuregulin receptor erb2b in neural and cardiac development. *Nature* 1995; 378: 394–398.

172 Kuramochi Y, Cote GM, Guo X, *et al.* Cardiac endothelial cells regulate reactive oxygen species-induced cardiomyocyte apoptosis through neuregulin-1beta/erb4 signaling. *J Biol Chem* 2004; 279: 51141–51147.

173 Lemmens K, Doggen K, De Keulenaer GW. Role of neuregulin-1/erb2 signaling in cardiovascular physiology and disease: implications for therapy of heart failure. *Circulation* 2007; 116: 954–960.

174 Geisberg CA, Wang G, Safa RN, *et al.* Circulating neuregulin-1beta levels vary according to the angiographic severity of coronary artery disease and ischemia. *Coron Artery Dis* 2011; 22: 577–582.

175 Barwari T, Joshi A, Mayr M. Circulating miRNA in cardiovascular disease. *J Am Coll Cardiol* 2016; 68: 2577–2584.

176 Wang GK, Zhu QJ, Zhang JT, *et al.* Circulating miRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J* 2010; 31: 659–666.

177 Karakas M, Schulte C, Appelbaum S, *et al.* Circulating miRNAs strongly predict cardiovascular death in patients with coronary artery disease-results from the large atherogene study. *Eur Heart J* 2016; pii: ehw250.

178 Bye A, Rosjo H, Nauman J, *et al.* Circulating micrornas predict future fatal myocardial infarction in healthy individuals—the HUNT study. *J Mol Cell Cardiol* 2016; 97: 162–168.

179 Zapetaki A, Willeit P, Tilling L, *et al.* Prospective study on circulating micrornas and risk of myocardial infarction. *J Am Coll Cardiol* 2012; 60: 290–299.