Novel Risk Markers and Risk Assessments for Cardiovascular Disease

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Abstract: The use of risk markers has transformed cardiovascular medicine, exemplified by the routine assessment of troponin, for both diagnosis and assessment of prognosis in patients with chest pain. Clinical risk factors form the basis for risk assessment of cardiovascular disease and the addition of biochemical, cellular, and imaging parameters offers further refinement. Identifying novel risk factors may allow greater risk stratification and a steady, but gradual progression toward precision medicine. Indeed, the generation of data in this area of research is explosive and when combined with new technologies and techniques provides the potential for more refined, targeted approaches to cardiovascular medicine. Although discussing the most recent developments in this field, this review article aims to strike a balance between novelty and validity by focusing on recent large sample-size studies that have been validated in a separate cohort in most cases. Risk markers related to atherosclerosis, thrombosis, inflammation, cardiac injury, and fibrosis are introduced in the context of their pathophysiology. Rapidly developing new areas, such as assessment of micro-RNA, are also explored. Subsequently the prognostic ability of these risk markers in coronary artery disease, heart failure, and atrial fibrillation is discussed in detail. (Circ Res. 2017;120:133-149. DOI: 10.1161/CIRCRESAHA.116.309955.)

Key Words: biomarkers ■ cardiovascular diseases ■ heart failure ■ risk ■ risk factors

The use of risk markers has transformed cardiovascular medicine, as exemplified by routine measurement of troponin, both for diagnosis and assessment of prognosis in patients with chest pain. There is a constant drive to identify novel risk markers that may allow for evermore accurate risk stratification, with the ultimate aim of directing treatments only to the patients who will benefit from them. Furthermore, identification of risk markers that are also causative in disease processes may unveil mechanisms that allow for the development of novel treatment strategies.

However, assessments of novel risk markers have been plagued by confounding factors, methodological limitations, and statistical dilemmas. Indeed, these difficulties have been well described in detailed review articles on the subject.1,2 This review article does not therefore attempt to provide an exhaustive list of risk markers; instead, the focus is on novel, well-validated markers from clinical studies with large sample sizes. Accordingly, ≈75% of the clinical studies in this review had a sample size of >1000 patients.

The first part of this review introduces potential risk markers in the context of their involvement in the pathophysiology of cardiovascular disease. This highlights many shared themes, including dyslipidemia, thrombosis, inflammation, fibrosis, and hemodynamic stress. Furthermore, this provides insight into why each marker may be associated with increased risk in cardiovascular disease. Next, this review discusses the relationship between each marker and cardiovascular risk in many different clinical contexts. Combined with an understanding of the pathophysiological role of each marker, this helps to suggest whether each marker could be a simple risk biomarker or may also represent a modifiable risk factor.

Overview of the Role of Risk Markers in the Pathophysiology of Cardiovascular Disease

Lipid-Related Risk Markers
Coronary artery disease is the leading cause of death worldwide. Atherosclerosis of the coronary arteries is primarily driven by cholesterol and, in particular, low-density lipoprotein cholesterol (LDL-C).3 The causative role of LDL-C in coronary artery disease is clearly demonstrated by the success of LDL-C-lowering drugs, such as statins, and Mendelian randomization studies.3,4 Recently, it has been discovered that proprotein convertase subtilisin/kexin type 9 has a major role in the regulation of LDL-C, and levels of soluble proprotein convertase subtilisin/kexin type 9 have been identified as a new marker of cardiovascular risk.5

Although traditionally thought to reduce cardiovascular risk, the role of high-density lipoprotein cholesterol has now been brought into question by negative findings from clinical trials of drugs that increase high-density lipoprotein cholesterol and by negative Mendelian randomization studies.3,4 In

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Platelet reactivity to various agonists, including ADP, has been studied extensively in the context of acute coronary syndrome (ACS; Table 2).11 The VerifyNow and Multiplate point-of-care tests allow for the measurement of platelet aggregation in response to stimulation, and it has been shown that high platelet reactivity is associated with adverse cardiovascular events.26 Platelet microparticles are released on platelet activation, and their role in cardiovascular disease is an area of active investigation (Table 2).15 Circulating microparticles are released from cells undergoing activation or apoptosis and are a type of small plasma membrane vesicle that retain defined properties from their original cell lineage.15

Platelet activation and aggregation lead to the activation of the coagulation cascade and the formation of a stable cross-linked fibrin clot. The balance between prothrombotic factors and endogenous fibrinolysis determines whether the thrombus propagates or instead proceeds to dissolution.27 Because thrombosis is central to the pathophysiology of ACS and embolic stroke in atrial fibrillation (AF), both prothrombotic factors and markers of endogenous fibrinolysis have been extensively investigated in these conditions (Table 2).17,27

### Inflammation-Related Risk Factors

Inflammation has a central role in the pathophysiology of many cardiovascular diseases, including coronary artery disease, heart failure, and AF. Mediators of inflammation have therefore been investigated in particular as potential risk markers. Coronary artery atherosclerosis is characterized by lipid-driven chronic inflammation. Macrophages, which have a key role in lipid handling, are the most numerous leukocyte population within the atherosclerotic plaque. After atherosclerotic plaque rupture, complex cross talk between platelets and leukocytes amplifies the development of a thrombus and the initiation of systemic inflammation.28,29

The thrombus may occlude the coronary artery, resulting in myocardial ischemia, which leads to the release of damage-associated molecular patterns, such as heat shock proteins. Damage-associated molecular patterns trigger leukocyte release of proinflammatory cytokines, which drives systemic inflammation and is characterized by increased levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and C-reactive protein (CRP; Table 2). Endothelial cells then upregulate adhesion molecules, which promotes neutrophil extravasation into myocardial tissue in response to chemotaxis toward released chemokines.30 Neutrophils phagocytose necrotic tissue and release their granules, which contain proteolytic enzymes and mediators such as myeloperoxidase, which contribute toward oxidative stress (Table 2).30 The subsequent proliferative phase involves many different cell populations, including monocytes, fibroblasts, and endothelial progenitor cells, and results in a transition from inflammation toward cardiac repair (Table 2).31 It has recently been identified that there are at least 3 different phenotypes of monocyte. Classical monocytes (Mon1), intermediate (Mon2), and nonclassical monocytes (Mon3) differ in their expression of the lipopolysaccharide coreceptor CD14 and the FcγRII receptor CD16.22 Gene expression profiling and function investigations have demonstrated distinct and often opposing roles of intermediate and nonclassical monocytes,32 which were previously

### Table 1. Selected Lipid-Related Risk Factors

<table>
<thead>
<tr>
<th>Marker</th>
<th>Released in Response to</th>
<th>Role</th>
</tr>
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<tbody>
<tr>
<td>PCSK9</td>
<td>Influenced by use of statins and diurnal variation</td>
<td>Promotes the degradation of hepatocyte LDL receptors, which have an important role in lowering plasma levels of LDL.9</td>
</tr>
<tr>
<td>OxPL</td>
<td>Oxidative stress</td>
<td>OxPL, which are covalently bound to lipoproteins that contain apoB&lt;sub&gt;48&lt;/sub&gt;. Suggested to be a specific biomarker for oxidation.5</td>
</tr>
<tr>
<td>Lp-PLA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Inflammation</td>
<td>Role in depletion of oxPLs from lipoproteins.7</td>
</tr>
<tr>
<td>sPLA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Inflammation</td>
<td>Hydrolyze LDL, which forms smaller, denser LDL particles that are more proatherogenic.8</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; Lp-PLA<sub>2</sub>, lipoprotein-associated phospholipase A2; OxPL, Oxidized phospholipids; PCSK9, proprotein convertase subtilisin/kexin type 9; and sPLA<sub>2</sub>, secretory phospholipase A2.
Table 2. Selected Risk MarkersRelated to Thrombosis and Inflammation

<table>
<thead>
<tr>
<th>Marker</th>
<th>Modified by</th>
<th>Role</th>
</tr>
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</table>
| Platelet reaction              | Antiplatelet therapy                 | Assessment of the reactivity of platelet signaling pathways can be used to identify response to antiplatelet therapy in the context of coronary artery disease. Results of the VerifyNow and Multiplate tests are well standardized and are predictive of adverse cardiovascular events.
|                                | Thrombosis                           |                                                                      |
| Platelet and erythrocyte       | Arterial thrombosis                  | Platelet-derived microparticles have a role in thrombosis and inflammation and interact with endothelial cells, via deposition of inflammatory and thrombotic mediators. Erythrocyte-derived microparticles are released from growing thrombi and increase the thrombin generation of blood. |
| microparticles                 | Inflammation Atherosclerosis          |                                                                      |
| D-dimer                        | Thrombosis                           | A fibrin-degradation product, which is a robust marker of thrombosis and subsequent fibrin degradation. Although the stability of a clot is critically dependent on the balance between thrombosis and endogenous fibrinolysis, there are few robust measures of endogenous fibrinolysis. |
| IL-6                           | Inflammation                         | Central role in cytokine signaling cascades, which lead to the production of CRP, fibrinogen and other acute phase reactants. IL-6 receptor pathways have a causative role in the development of coronary artery disease. |
| CRP                            | Inflammation                         | Produced by hepatocytes in response to circulating cytokines, particularly IL-6. Robust downstream marker of inflammation, although unlikely to have a causative role in cardiovascular disease. |
| MPO                            | Inflammation                         | Contained within the granules of leukocytes, particularly neutrophils, and catalyzes the conversion of H₂O₂ to reactive oxygen species on release. Reactive oxygen species suppress nitric oxide and impair the function of many proteins because of oxidative post-translational modifications. |
| Monocyte phenotype             | Inflammation ACS                     | Recently recognized that there are 3 distinct phenotypes of monocyte, defined as Mon1 (classical CD14++CD16−), Mon2 (intermediate CD14++CD16−), and Mon3 (nonclassical CD14++CD16−). Each phenotype differs in antigen presenting capacity, phagocytic activity and cytokine generation. |
| Circulating progenitor cells   | Inversely associated with cardiovascular risk factors | Immature bone marrow-derived cells, which are mostly of hematopoietic origin. Involved in endothelial repair and angiogenesis. |
| Leukocyte and endothelial      | Inflammation Thrombosis Atherosclerosis | Plasma levels of endothelial microparticles are thought to be associated with chronic endothelial dysfunction and vascular injury. |
| microparticles                 |                                      |                                                                      |

ACS indicates acute coronary syndrome; CRP, C-reactive protein; and IL, interleukin.

Risk Markers Related to Cardiac Injury, Healing, and Fibrosis

During acute and chronic cardiac injury, reparative mechanisms must find a careful balance between remodeling the heart to maintain structural integrity while also preserving myocardial function. Acute cardiac injury induces myocardial infiltration of leukocytes and proliferation of resident fibroblasts, which differentiate into myofibroblasts. The initial inflammatory response is then suppressed, in part, by anti-inflammatory cytokines, and transforming growth factor (TGF)-β activates myofibroblasts, which promotes collagen synthesis. It is clear that TGF-β has a major role in the formation of a collagenous scar and myocardial fibrosis although the exact regulatory role of each TGF-β isoform remains difficult to establish. Interestingly, however, nonspecific suppression of TGF-β signaling invariably causes mortality because of myocardial rupture in animal models of myocardial infarction (MI). In addition, neutrophils and macrophages release matrix metalloproteinases (MMPs), such as MMP9, which degrade extracellular matrix components and are regulated by complex cross talk with TGF-β. Raised levels of growth differentiation factor (GDF)-15 in patients with MI and AF could reflect a poor prognosis related to the need to activate a protective anti-inflammatory pathway (Table 3). Alternatively, in the case of MMP9, ST2, and galectin-3, raised levels could represent immune dysregulation and a propensity toward cardiac fibrosis and subsequent heart failure (Table 3). Cardiac fibrosis also has a major role in the pathophysiology of AF. Fibroblast proliferation within the myocardium and the formation of scar tissue may both contribute toward heterogeneity of impulse conduction, thereby predisposing toward arrhythmia.

Risk Markers Related to Hemodynamic Stress and Renal Function

Mechanical stretch and other mediators, such as angiotensin II and adrenergic agonists, induce cleavage of preprohormones and secretion of natriuretic peptides by cardiomyocytes. Both atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) predominantly act on natriuretic peptide receptor A, which leads to the inhibition of the renin–angiotensin–aldosterone system, diuresis, and natriuresis.
ANP and BNP is both increased during hemodynamic overload and cardiac remodeling and are therefore indicative of heart failure (Table 3). Additional markers of hemodynamic stress and renal disturbance include copeptin and cystatin-C, respectively (Table 3).

### Micro-RNA–Related Risk Markers

There has been much recent interest in the role of micro-RNAs (miRNAs) in the pathophysiology of cardiovascular disease. miRNAs are short noncoding RNAs that have a major role in pathophysiological stress responses and mediate many cellular processes by regulating gene expression. miRNAs interact with specific mRNA to regulate their translation, largely by suppression of protein synthesis. Most miRNAs are located intracellularly while platelets and platelet microparticles are an abundant circulating source. miRNAs regulate a diverse range of processes in cardiovascular disease, including myocardial remodeling and fibrosis, vascular inflammation, lipid processing, and electric remodeling (Table 5). It has also recently been shown that miRNAs can act as a novel biomarker for platelet reactivity and that their levels can be manipulated by the administration of antiplatelet therapy. Specific synthetic antagonists of miRNAs (antagomiRs) are currently in development, and it is possible that these could prove beneficial in cardiovascular disease.

In recent years, there has been a dramatic increase in the number of imaging modalities that are available for use in clinical practice. Cardiac imaging provides sophisticated measures of many different pathological processes, including quantification of coronary artery disease and the presence of plaque rupture, as well as determination of myocardial infarct size and the presence of microvascular obstruction (Table 5). More recent techniques, such as coronary computed tomographic angiography and optical coherence tomography, allow...
for some assessment of plaque morphology, beyond simple assessment of luminal stenosis (Table 5). Further development of imaging technologies may offer the possibility for detailed plaque assessment, including determination of plaque inflammation, which may suggest plaques at high risk of rupture.

**Risk Markers for Developing Cardiovascular Disease**

### Clinical Factors and Risk Scores

Clinical factors, such as age, hypertension, diabetes mellitus, hyperlipidemia, and family history, are still the predominant indicators for likelihood of developing coronary artery disease, which has classically been assessed using the Framingham risk score. The Reynolds risk score, which incorporates CRP, may provide additional predictive capacity compared with the Framingham risk score.

### Lipids

It has long been recognized that the level of LDL-C is a major risk factor for the development of coronary artery disease. In the Bruneck study, mass spectrometry demonstrated that certain species of triacylglycerols, cholesterol esters, and phosphatidylethanolamines have a strong predictive value for the development of cardiovascular disease. Mass spectrometry has also shown that sphingolipids, phospholipids, cholesterol esters, and glycerolipids are associated with an increased risk of adverse cardiovascular events in patients with diabetes mellitus.

High-throughput metabolite profiling by nuclear magnetic resonance spectroscopy has also identified a further 33 lipids and metabolites that are predictive of cardiovascular events, although only 4 metabolites showed independent risk of adverse cardiovascular events when routine lipids were adjusted for, phenylalanine and monounsaturated fatty acid levels were associated with increased risk whereas omega-6 fatty acids and docosahexaenoic acid were associated with decreased risk. Novel lipid-related markers, including serum levels of proprotein convertase subtilisin/kexin type 9, oxidized phospholipids, and secretory phospholipase A (Table 1), have also recently been shown to be associated with a risk of developing coronary artery disease in the general population.

### Inflammation

Inflammation has a central role in the pathophysiology of atherosclerosis, which is highlighted by the high cardiovascular risk of systemic inflammatory disorders, particularly systemic autoimmune disorders and systemic vasculitis. In the general population, high-sensitivity (hs) CRP and fibrinogen both provide additive predictive ability for cardiovascular disease although the incremental addition beyond clinical risk factors is relatively modest.

More recent, but less clearly established, emerging predictors of coronary artery disease in the general population include TNFRII, EN-RAGE, and soluble CD14. A lesser effect on the risk of coronary heart disease events.

As some of these markers are predictive of asymptomatic structural heart disease, it is not known whether they are predicting the development of heart failure or identifying subclinical heart failure. In the general population, GDF-15 is also associated with an increased risk of cancer and all-cause mortality, thus demonstrating that it acts as a broad marker of disease.

### Genetics

The heritability of coronary artery disease has been estimated to be as high as 40% to 50% on the basis of twin and family studies. Currently, 45 risk alleles have been identified to confer risk of coronary artery disease. Network analysis has demonstrated that many of the related genes have an important role in lipid metabolism and inflammation, emphasizing the causal role of these processes in the development of coronary artery disease. Genome-wide association studies have identified many single-nucleotide polymorphisms that are associated with an increased risk of coronary heart disease. Based on these polymorphisms, genetic risk scores are able to modestly improve prediction of coronary heart disease, peripheral arterial disease, and sudden cardiac death beyond established risk factors in the general population.

The modest incremental value of genetic factors may be because adverse genotypes are related to dyslipidemia, dysglycemia, and hypertension, which are already accounted for by traditional risk factors. In addition, genetic risk may be related to complex intergene interactions that have not yet been identified. Although it has been shown that genetic testing can potentially be used to guide treatment in patients with familial hypercholesterolemia, the use of genetic testing in other settings, such as primary prevention, is not currently recommended. miRNAs are small noncoding RNAs that regulate gene expression (Table 4). In the general population, miRNA-126 is positively correlated with the risk of future coronary events (adjusted hazard ratio, 2.69), whereas miR-223 and miR-197 are inversely associated with the risk of future coronary events.

### Imaging

Coronary artery calcium score and carotid plaque burden are associated with an increased risk of the development of adverse cardiovascular events in the general population (Table 5), independently of clinical markers and inflammatory biomarkers. Conversely, a calcium artery score of 0 has a strong negative predictive value for the development of coronary artery disease.

### High-Risk Groups Within the General Population

In the general population, dysglycemia and diabetes mellitus are associated with upregulation of numerous inflammatory and prothrombotic pathways and have a direct role in the causation of coronary artery disease. Screening of ≥280 biomarkers in patients with dysglycemia showed positive associations between NT-proBNP, trefoil factor 3, GDF-15, apolipoprotein B, angiopoietin-2, osteoprotegerin and α-2-macroglobulin, and adverse cardiovascular events. Conversely, hepatocyte growth factor receptor, glutathione S transferase α, and chromogranin A were inversely associated with adverse cardiovascular events. NT-proBNP has also
been positively associated with cardiovascular risk in another study that additionally demonstrated an association of osteopontin and MMP-3 with adverse cardiovascular events.\textsuperscript{114}

### Risk Markers for Adverse Cardiovascular Events in Patients With Stable Coronary Artery Disease

#### Clinical Risk Markers

In patients with stable coronary artery disease, the main determinants of risk of developing an ACS are related to sociodemographic characteristics, pattern of coronary artery disease progression, traditional risk factors, cardiovascular and non-cardiovascular comorbidities, heart rate, and levels of creatinine, hemoglobin, and white cell count.\textsuperscript{115}

#### Additional Risk Markers in Patients With Stable Coronary Artery Disease

In patients with stable coronary artery disease, markers of cardiac injury and hemodynamic stress (Table 5), such as cTnT, NT-proBNP, MR-proANP, and MR-proADM (midregional pro-adrenomedullin), are associated with an increased risk of cardiovascular events and death.\textsuperscript{116–118} However, even though raised levels of cTnT are an independent predictor of adverse cardiovascular events in patients with diabetes mellitus and stable coronary artery disease, this risk is not reduced by prompt coronary revascularization.\textsuperscript{116} This could be because the pathophysiology of troponin release in stable coronary artery disease (related to chronic small vessel ischemia, hypertension, and metabolic abnormalities) may be relatively unresponsive to coronary revascularization.\textsuperscript{116}

High platelet reactivity despite treatment with dual-antiplatelet therapy is associated with an increased risk of adverse cardiovascular events in patients undergoing percutaneous coronary intervention.\textsuperscript{119} It has recently been shown that immature platelets are associated with an increased risk of adverse cardiovascular events\textsuperscript{120} and higher levels of platelet reactivity in patients with stable coronary artery disease.\textsuperscript{121} Established markers of inflammation (Table 2), such as hsCRP, are associated with adverse cardiovascular events in patients with stable coronary artery disease.\textsuperscript{122} In addition, emerging novel inflammatory risk markers include heat shock protein 70,\textsuperscript{122} amyloid β-peptide,\textsuperscript{123} and levels of circulating progenitor cells.\textsuperscript{124} miRNA are small noncoding RNA with many regulatory roles in the pathophysiology of cardiovascular disease (Table 4). Changes in levels of miR-126, miR-145, miR-155, and miR-29b are associated with plaque burden as determined by optical coherence tomography in patients undergoing elective angiography.\textsuperscript{125}

<table>
<thead>
<tr>
<th>Table 4. Selected Cardiovascular miRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
</tr>
<tr>
<td>miR-1</td>
</tr>
<tr>
<td>miR-133</td>
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<tr>
<td>miR-208</td>
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<tr>
<td>miR-499</td>
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<tr>
<td>miR-150</td>
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<tr>
<td>miR-223</td>
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<tr>
<td>miR-197</td>
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<tr>
<td>miR-126</td>
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<td>miR-143/145</td>
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<td>miR-155</td>
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<td>miR-622</td>
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<tr>
<td>miR-21</td>
</tr>
<tr>
<td>miR-29</td>
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<tr>
<td>miR-328</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; AF, atrial fibrillation; BNP, brain natriuretic peptide; MI, myocardial infarction; MMP, matrix metalloproteinase; and VSMC, vascular smooth muscle cell.
### Table 5. Selected Imaging-Related Risk Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Released in Response to</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarct size (CMR)</td>
<td>Myocardial infarction</td>
<td>Demonstrated by infarcted myocardial tissue in late gadolinium images on CMR imaging.102</td>
</tr>
<tr>
<td>Microvascular obstruction (CMR)</td>
<td>Microvascular thrombosis and inflammation</td>
<td>Demonstrated by regions of myocardial hypoenhancement during the first 2 min of gadolinium contrast administration.102</td>
</tr>
<tr>
<td>CTCA</td>
<td>Coronary artery disease</td>
<td>Noninvasive investigation for detecting obstructive and nonobstructive coronary artery disease, which allows rapid determination of the diagnosis of suspected angina.103 CTCA correlates well with the findings of invasive angiography, with high accuracy and sensitivity of &gt;97% in several studies104 although this may be lower in distal lesions. Coronary artery calcium scoring provides an indicator of the overall prevalence of coronary artery disease.71</td>
</tr>
<tr>
<td>MR coronary angiography</td>
<td>Coronary artery disease</td>
<td>MR coronary angiography is appealing as it does not involve radiation and provides excellent soft-tissue contrast.105 However, current applications of MR coronary angiography are limited because of temporal resolution, which is exacerbated by the small caliber and complex motion of the coronary arteries.105</td>
</tr>
<tr>
<td>Optical coherence tomography</td>
<td>Plaque rupture</td>
<td>Intracoronary light-based technology that allows for detailed characterization of coronary artery plaque. Plaque rupture can be differentiated from an intact fibrous cap on the basis of a discontinuity in the fibrous cap.106</td>
</tr>
</tbody>
</table>

CMR indicates cardiac magnetic resonance; CTCA, CT coronary angiography; and MR, magnetic resonance.

### Risk Markers for Prognosis After ACSs

The pathogenesis of ACS is primarily driven by atherosclerosis, thrombosis (Table 2), inflammation (Table 2), and cardiac injury (Tables 4 and 5), with subsequent deterioration in myocardial function (Figure 1).

### Clinical Factors and Risk Scores

In patients with ACS, the Global Registry of Acute Coronary Events score (based on age, heart rate, blood pressure, Killip class [which is related to the development of heart failure], creatinine, ST-segment deviation, cardiac arrest, and elevated troponin) is the most clearly established score for determining risk of adverse cardiovascular events.126 Conversely, the Can Rapid Risk Stratification of Unstable Angina Patients Suppress Adverse Outcomes With Early Implementation of the ACC/AHA Guidelines score (based on hematocrit, renal function, heart rate, blood pressure, previous vascular disease, diabetes mellitus, heart failure, and sex) has been established as predictive of bleeding.127

More recent risk scores from the PARIS registry (Patterns of Non-Adherence to Anti-Platelet Regimens in Stented Patients) have been developed to determine patients who are at high risk of out-of-hospital coronary thrombotic events (based on diabetes mellitus, type of ACS, smoking, renal function, and

![Figure 1. Acute coronary syndromes. A. Foam cells (derived from macrophages [Ma]) and lymphocytes have a central role in the development of a lipid-rich atherosclerotic plaque with a necrotic core (NC). Rupture or erosion of an atherosclerotic plaque triggers platelet (P) adhesion to subendothelial components, resulting in the formation of an occlusive thrombus, which also recruits monocytes (M) and neutrophils (N). Platelet–leukocyte interactions cause the release of proinflammatory cytokines and recruited neutrophils also release neutrophil extracellular traps. B. Myocardial ischemia, caused by coronary artery obstruction, leads to the recruitment of neutrophils and monocytes toward chemokines. Leukocyte adhesion molecules then mediate transmigration of leukocytes. Monocytes may then differentiate into Ma, alongside Ma that are already resident within myocardial tissue. Fibroblasts (F) proliferate and differentiate into myofibroblasts (MF). C. Impaired myocardial contractility and hemodynamics results in myocardial stretch, leading to consequent renal disturbances.](http://circres.ahajournals.org/)
previous revascularization or at high risk of major bleeding (based on age, body mass index, smoking, anemia, renal function, and whether on an anticoagulant in addition to antiplatelet therapy). In patients considered for long-term dual antiplatelet administration, the DAPT score (dual antiplatelet therapy; based on age, smoking, diabetes, MI at presentation, prior MI or percutaneous coronary intervention, stent type and diameter and heart failure) may provide a way of categorizing patients who are likely to receive a favorable net benefit from long-term DAPT, related to high ischemic risk and lower bleeding risk.

Platelets and Thrombosis

Antiplatelet therapy, particularly clopidogrel, has a variable effect on platelet reactivity (Table 2) during treatment for ACS. It is now well established that high levels of platelet reactivity during treatment with clopidogrel are associated with an increased incidence of adverse cardiovascular events. However, increasing the dose of clopidogrel in patients with high residual platelet reactivity has not been shown to reduce this risk of adverse cardiovascular events. Furthermore, high platelet reactivity is now relatively rare in patients treated with more recent antiplatelet agents, such as prasugrel and ticagrelor. Consequently, it is not recommended to routinely assess the effect of clopidogrel and aspirin on platelet reactivity. It has more recently been shown that very low levels of on-treatment platelet reactivity are associated with an increased risk of bleeding although it is unclear how this should best be managed.

The development of a thrombus after the rupture of an atherosclerotic plaque may also be dependent on a prothrombotic state with failure of spontaneous endogenous thrombolysis. Key regulators of fibrinolysis include tPA (tissue-type plasminogen activator), plasminogen activator inhibitor-1, α-2 antiplasmin, α-2-antiplasmin–plasmin complex, D-dimer, soluble fibrin, thrombin activatable fibrinolysis inhibitor, and Lp(a). The balance between thrombosis and fibrinolysis can be assessed using thromboelastography, rotational thromboelastometry, or the Global Thrombosis Test. However, there are technical limitations for many of these tests, and data on their prognostic capabilities are in many cases conflicting. Recently, it has been shown that levels of coagulation factor XIII decrease during acute MI, and the magnitude of the decrease is associated with subsequent development of heart failure, adverse cardiovascular events, and death.

Inflammation

It is well-established that elevated levels of inflammatory mediators (Table 2), such as hsCRP, are associated with an increased risk of adverse cardiovascular events in patients with ACS. More recently, novel inflammatory mediators, many of which are related to neutrophils, have been linked to cardiovascular risk in patients with ACS. Neutrophil gelatinase-associated lipocalin is stored in neutrophil granules and is released by damaged tubular cells and may also have a role in atherosclerosis. Increased levels of neutrophil gelatinase-associated lipocalin are associated with an increased risk of all-cause mortality and adverse cardiovascular events in patients with ST-segment–elevation MI. Neutrophil gelatinase-associated lipocalin forms a complex with MMP9 and prevents its degradation. Levels of this complex may be predictive of adverse cardiovascular events in patients with ACS undergoing coronary angiography. Neutrophils release neutrophil extracellular traps, which consist of extruded DNA, during ACS, and neutrophil extracellular traps contribute toward the meshwork of a thrombus. The intracoronary burden of neutrophil extracellular traps is positively associated with myocardial infarct size. Conversely to the apparently deleterious role of neutrophils, circulating progenitor cells may have a protective role during ACS. It has recently been established that levels of CD34+CD133+ circulating progenitor cells are inversely associated with adverse cardiovascular events and all-cause mortality in patients with ACS or undergoing angiography. However, it must be cautioned that some circulating endothelial progenitor cells assays are relatively nonspecific as proteomics has shown that platelet microparticles can cause the transfer of endothelial characteristics to mononuclear cells. During ACS, it has been identified that there is differential mobilization of each of the 3 phenotypes of monocyte (Table 2), but it has not yet been identified how this influences prognosis.

Cardiac Injury

The routine measurement of troponin (Table 3) has revolutionized the diagnosis and risk stratification of patients presenting acutely with chest pain. In patients presenting with symptoms suggestive of ACS, the risk of mortality is low in patients with hs-cTnT <12 ng/L (≈2%), and there is a progressive increase in risk of mortality from ≈2% to ≈16% to 20% in patients with hs-cTnT between 12 and 62 ng/L. The risk of mortality then seems to plateau at ≈16% to 20% for patients with hs-cTnT >62 ng/L. In patients with established ACS, higher levels of cTnT are associated with an increased risk of cardiovascular death, with little impact on the risk of a recurrent MI.

It has also been investigated whether markers of hemodynamic stress (Table 5), such as copeptin, may also prove useful in the diagnosis of acute chest pain. Although copeptin is predictive of MI in patients presenting with chest pain, it is less predictive than troponin. There is evidence that the combination of cTnI and copeptin provides a greater negative predictive value than cTnI alone, however. It could, therefore, be investigated whether this allows immediate rule out of MI, without the need for serial blood sampling.

Cardiac Stress, Fibrosis, and Renal Function

Plasma levels of ST2, galectin-3, GDF-15, NT-proBNP, MR-proANP, and MR-proADM are associated with an increased risk of cardiovascular events, particularly related to heart failure, in patients with ACS. Although the majority of these markers seem to mostly reflect the risk related to heart failure, NT-proBNP was associated with increased risk of both cardiovascular death and spontaneous MI in the PLATO study (Platelet Inhibition and Patient Outcomes). Integrating GDF-15 with the Global Registry of Acute Coronary Events score improves prediction of adverse cardiovascular events in patients with non-ST-segment–elevation-ACS. However, in addition to its association with adverse cardiovascular events, GDF-15 was also associated with the risk of major bleeding in the PLATO study.

Although levels of cystatin C are associated with adverse cardiovascular events in patients with ACS, use of cystatin C does not seem to outperform measurements based on creatinine.
More recently established markers of cardiovascular risk related to hemodynamic stress also include plasma levels of proenkephalin and soluble corin.

**Imaging**

Novel imaging modalities (Table 5) also allow greater risk stratification of patients with ACS. Plaque rupture identified on optical coherence tomography is an independent predictor of adverse cardiovascular events in patients with ACS undergoing coronary angiography. In addition, left ventricular ejection fraction, infarct size, and microvascular obstruction as determined by cardiac magnetic resonance are all predictors of adverse cardiovascular events in patients with ST-segment–elevation MI.

**Cardiac Injury and Stress**

Cardiac injury and stress, natriuretic peptides (Table 3), inflammation (Table 2), and fibrosis (Table 3) all have important roles in the pathophysiology of heart failure (Figure 2).

**Clinical Factors and Risk Scores**

In a large meta-analysis of 39,372 patients, the most significant predictors of mortality in patients with heart failure were identified to be age, lower ejection fraction, New York Heart Association class, creatinine, diabetes mellitus, absence of β-blocker treatment, lower systolic blood pressure, lower body mass, time since diagnosis, smoking, chronic obstructive pulmonary disease, male sex, and absence of angiotensin-converting enzyme inhibitor or angiotensin-receptor blocker treatment. Incorporation of these into the MAGGIC (Meta-Analysis Global Group in Chronic Heart Failure trial) risk score allows assessment of risk of mortality in patients with heart failure. The EMPHASIS HF (Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure trial) risk score (based on age >75 years, male sex, systolic blood pressure, renal function, diabetes mellitus, previous heart failure hospitalization, anemia, previous MI/CABG (coronary artery bypass graft surgery), low body mass index, and elevated heart rate) is predictive of hospitalization for heart failure or cardiovascular death in patients with heart failure and mild symptoms. Use of these risk scores could have the potential to aid treatment decisions, as patients with higher levels of risk seem to derive more absolute benefit from intensification of treatment for heart failure.

**Cardiac Injury and Stress**

Hs-cTnT (Table 3) levels are associated with mortality in patients admitted with decompensated heart failure and anatriuretic peptides (Table 3). Atrial fibrillation (AF) can be precipitated by a variety of insults, including abnormal calcium homeostasis and ion-channel dysfunction, play a particularly prominent role in precipitating AF, whereas inflammation and oxidative stress reinforce pathological changes in myocardial structure. After the onset of AF, reduced blood flow through the atria predisposes toward thrombosis. The formation of an atrial thrombus, with subsequent embolization to the brain, is one of the most important causes of stroke, which may have devastating consequences. It is well-recognized that thrombosis may not purely be related to stasis of blood within the atria, but likely also reflects multiple clinical risk factors for stroke, which are particularly common in patients with AF.
increased risk of adverse cardiovascular events in patients with chronic heart failure.149 Patients with heart failure and recovery of left ventricular function tend to have a lower level of TnI than patients who have not recovered left ventricular function.150 ST2 and galectin-3 have been proposed to be markers related to inflammation and myocardial fibrosis (Table 3). Levels of ST2 are independently associated with adverse cardiovascular events and mortality, both in patients admitted with decompensated heart failure and in patients with chronic heart failure.148,149,151 Galectin-3 is associated with measures of heart failure severity and all-cause mortality in patients with chronic heart failure although this association does not seem to be independent of the effect of NT-proBNP.152 In comparison to ST2, galectin-3 seems to be more closely related to all-cause mortality, rather than to cardiovascular death specifically, and galectin-3 has a lesser ability to improve discrimination and reclassify risk of mortality in patients with heart failure.153 GDF-15, which is proposed to be a marker of inflammation and oxidative stress (Table 3), is independently associated with adverse cardiovascular events in patients with chronic heart failure.148

MMPs and collagen processing have an important role in myocardial fibrosis. C-terminal telopeptide of collagen type I: MMP-1 ratio has been identified as a possible new biomarker of adverse collagen processing in patients with chronic hypertensive heart failure and is associated with hospitalization for heart failure.150 Emerging novel markers of risk in patients with heart failure relating to cardiac injury and stress also include levels of soluble neprilysin,154 soluble FLT-1 (Fms-related tyrosine kinase 1),150,155,156 and secretoneurin.157

Natriuretic Peptides
Hemodynamic overload during heart failure causes derangement of natriuretic peptides (Table 5). Patients with heart failure and a reduced ejection fraction have higher levels of NT-proBNP and BNP than patients with a preserved ejection fraction.158,159 In addition, it has recently been shown that low levels of BNP are predictive of recovery of left ventricular function in patients with heart failure.150,160 In patients with heart failure, increases in MR-proANP and copeptin are associated with an increased risk of death or transplantation although the effect of copeptin does not seem to be independent of other biomarkers and clinical parameters.148,161 MR-proADM is an independent predictor of mortality in patients admitted with decompensated heart failure.148,150

Inflammation
Although it has been established that inflammation has an important role in the pathophysiology and prognosis of acute heart failure, the role of inflammation in chronic heart failure is far less clearly defined.34 It is well-recognized that proinflammatory cytokines, particularly TNF-α, are upregulated in patients with chronic heart failure. Furthermore, animal models have demonstrated that proinflammatory cytokines, including IL-6 and TNF-α (Table 2), are increased during heart failure progression.34 Demonstrating this in humans, hsCRP is independently associated with mortality in patients admitted with decompensated heart failure.148 However, negative results from clinical trials of treatments targeting inflammation cast doubt over the issue of whether these inflammatory pathways are causal.34

Animal models have shown that activation of monocytes, macrophages, and dendritic cells is central to cardiac remodeling during chronic heart failure.162 Levels of the Mon2 monocye population (also known as intermediate monocytes) seem to be most closely linked with prognosis in patients with acute heart failure although it is not known whether their role is pathological or simply a marker of disease severity.163

Micro-RNA
Heart and muscle-specific miRNAs, including miR-1, miR-133, miR-208, and miR-499 (Table 4) are upregulated in advanced heart failure, which is associated with increased levels of troponin.164 Interestingly, deranged levels of these miRNAs almost completely resolve after the initiation of LV assist device support.164 miR-520d-5p, miR-558, and miR-122 have also been shown to be upregulated in patients with heart failure compared with controls,97 and levels of miR-622, miR-520d-5p, miR-519, and miR-200b inversely correlate with LV function as assessed by echocardiography.97

Risk Markers in AF
The pathophysiology of AF involves a complex interplay between patient comorbidities, electric abnormalities, structural heart disease, cardiac injury, inflammation (Table 2), oxidative stress (Table 2), and thrombosis (Table 2; Figure 3).

Clinical Risk Scores
In AF, there is an increasing focus on identifying patients who are at truly low risk of stroke, to definitively determine which patients may or may not require oral anticoagulation. Guidelines recommend the use of the CHA2DS2-VASC score (based on congestive heart failure, hypertension, age, diabetes mellitus, previous stroke, previous vascular disease, and sex) for stratifying stroke risk in patients.165,166 Analysis of the Swedish Atrial Fibrillation cohort study demonstrated that patients with a CHA2DS2-VASC score of 0 have a very low risk of stroke of ≈0.2%/y and a negative predictive value of 0.997.167 As with any clinical risk factor-based score, the predictive value is modest and can be improved by the addition of biomarkers. For example, the novel age, biomarkers, clinical history stroke score makes use of NT-proBNP and hs-cTnT (Table 3) in addition to age and previous history of stroke.168 Although the addition of biomarkers may offer additional predictive capability for identifying high-risk patients compared with clinical risk factor-based scores, their use is more complicated and expensive. Given that the default is to offer stroke prevention unless the patient is truly low risk, clinical decision making is simplified once the low-risk patients are identified, most simply by clinical scores (eg, CHA2DS2-VASC score of 0 in men, 1 in women). It is important that new biomarker-based risk scores should also aim to prioritize maximizing sensitivity for predicting subsequent stroke to ensure that patients at risk are initiated on anticoagulation appropriately.

For prediction of bleeding in anticoagulated AF patients, guidelines have recommended focusing on reversible bleeding risk factors, such as those incorporated into the HAS-BLED score (which is based on hypertension, renal and liver function, previous stroke, bleeding predisposition, labile international normalized ratios if on warfarin, age, concomitant medications, and the use of alcohol).165,166 Again, clinical factor-based
scores generally have a modest predictive value for bleeding, with C statistics of \( \approx 0.6 \).\textsuperscript{167,169–172} Bleeding risk is not static, and the appropriate use (and misuse) of bleeding risk scores has recently been discussed.\textsuperscript{173} The addition of biomarkers improves bleeding risk prediction. For example, the novel age, biomarkers, clinical history bleeding score incorporates the use of GDF-15 and hs-cTnT, in addition to hemoglobin, age, and previous bleeding.\textsuperscript{75} This provides additional predictive capability when compared with clinical risk factors alone but still with a modest C statistic of \( \approx 0.67 \) to 0.71 although it will not be possible to make full use of this score unless GDF-15 becomes routinely available in clinical practice.\textsuperscript{172}

### Cardiac Injury, Hemodynamic Stress, and Renal Function

The pathophysiology of AF is complex, and it is well recognized that many stressors of cardiac physiology (Tables 2 and 3) are linked with the development of AF. Approximately 75% of patients have levels of hsTnT higher than 7.5 ng/L.\textsuperscript{174} Higher levels of hsTnT are associated with an increased risk of stroke or systemic embolism and improve risk prognostication beyond the use of clinical variables alone.\textsuperscript{171} In particular, patients with persistently raised levels of hsTnT have a higher risk of stroke than patients with a transient rise in hsTnT.\textsuperscript{173} In patients with AF, a high proportion of patients also have increased levels of NT-proBNP, and the level of NT-proBNP is independently associated with an increased risk of stroke and MI.\textsuperscript{176} Again, patients with persistently raised NT-proBNP have a higher risk of stroke than patients with a transiently raised NT-proBNP.\textsuperscript{175} In patients presenting with a stroke, there is some evidence that levels of BNP can also be used to detect possible underlying asymptomatic AF.\textsuperscript{177} GDF-15 is associated with both an increased risk of stroke and an increased risk of bleeding in patients with AF, which may complicate its use for making decisions about the use of anticoagulation.\textsuperscript{178} Renal impairment, as indicated by the level of cystatin C, is also an independent predictor of adverse cardiovascular events in patients with AF.\textsuperscript{179,180}

### Inflammation and Coagulation

Although there are conflicting data, many studies have demonstrated higher levels of inflammatory markers, including CRP, and proinflammatory cytokines, such as IL-2, IL-6, IL-8, monocyte chemotactic protein-1 and TNF-\( \alpha \) in patients with AF compared with controls.\textsuperscript{181,182} IL-6 is independently associated with stroke and systemic embolism, major bleeding, and cardiovascular death in patients with AF.\textsuperscript{181,183} Similarly, CRP is independently associated with stroke or systemic embolism and cardiovascular death in patients with AF.\textsuperscript{181,183} It has also been shown that proinflammatory cytokines are predictors of the incidence of AF after cardioversion and surgery.\textsuperscript{181} In addition, levels of the fibrin-degradation product D-dimer, which are indicative of both inflammation and a prothrombotic state, are associated with an increased risk of stroke or systemic embolism and bleeding and mortality in patients with AF.\textsuperscript{184,185}

### Imaging

In patients undergoing catheter ablation for AF, atrial fibrosis determined by delayed enhancement cardiac magnetic resonance is independently associated with the recurrence of AF.\textsuperscript{186} Patients with persistent AF also have a greater degree of atrial fibrosis on cardiac magnetic resonance than patients with paroxysmal AF.\textsuperscript{187}

### Conclusions and Novel Approaches

Risk in cardiovascular disease is still determined predominantly by clinical factors. However, biochemical, cellular, and imaging parameters are steadily allowing for incrementally refined risk assessment. Over time, this is gradually moving us nearer to the paradigm of targeted, precision medicine\textsuperscript{188} although achieving this ideal will be difficult.

Looking ahead, systems biology approaches may allow for the assessment of multiple pathways simultaneously, as opposed to the assessment of individual pathways. Analysis of data in terms of complex networks may help to identify the key disturbances in underlying regulatory mechanisms. Furthermore, assessment of the trajectory of disease, over multiple time points, may allow for targeting of therapy to patients who are not responding in an optimal manner.

It is now clear that high-throughput techniques, such as genomics, proteomics, and metabolomics, will become ubiquitous. The quantity of data that is produced by these techniques is overwhelming. This will drive the use of computational biology and advanced statistical techniques, such as machine learning, so that our ability to understand data can catch up with our ability to generate data.

### Disclosures

Dr Lip has served as a consultant for Bayer/Janssen, BMS/Pfizer, Biotronik, Medtronic, Boehringer Ingelheim, Microlife and Daiichi-Sankyo. Speaker for Bayer, BMS/Pfizer, Medtronic, Boehringer Ingelheim, Microlife, Roche and Daiichi-Sankyo. Dr Thomas reports no conflicts.

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Correction to: Novel Risk Markers and Risk Assessments for Cardiovascular Disease

In the article by Thomas and Lip, “Novel Risk Markers and Risk Assessments for Cardiovascular Disease,” which published in the January 6th issue of the journal (Circ Res. 2017;120:133–149. DOI: 10.1161/CIRCRESAHA.116.309955), a correction was needed.

The authors inadvertently misstated the results of a recent study. The error has been corrected as follows: “The intracoronary burden of neutrophil extracellular traps is positively associated with myocardial infarct size.”

The authors apologize for the error.

This correction has been made to the current online version of the article, which is available at http://circres.ahajournals.org/content/120/1/133.