The influence of low-grade inflammation on platelets in patients with stable coronary artery disease

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Summary
Inflammation is likely to be involved in all stages of atherosclerosis. Numerous inflammatory biomarkers are currently being studied, and even subtle increases in inflammatory biomarkers have been associated with increased risk of cardiovascular events in patients with coronary artery disease (CAD). Low-grade inflammation may influence both platelet production and platelet activation potentially leading to enhanced platelet aggregation. Thrombopoietin is considered the primary regulator of platelet production, but it likely acts in conjunction with numerous cytokines, of which many have altered levels in CAD. Previous studies have shown that high-sensitive C-reactive protein (CRP) independently predicts increased platelet aggregation in stable CAD patients. Increased levels of CRP, fibrinogen, interleukin-6, stromal cell-derived factor-1, CXC motif ligand 16, macrophage migration inhibitory factor, RANTES, calprotectin, and copeptin have been associated with increased risk of cardiovascular events in CAD patients. Additionally, some of these inflammatory markers have been associated with enhanced platelet activation and aggregation. However, CRP and other inflammatory markers provide only limited additional predictive value to classical risk factors such as smoking, blood pressure, and cholesterol levels. Existing data do not clarify whether inflammation simply accompanies CAD and increased production and aggregation of platelets, or whether a causal relationship exists. In this review, we provide a comprehensive overview of inflammatory markers in stable CAD with particular emphasis on platelet production, activation, and aggregation in CAD patients.

Keywords
Inflammation, platelet aggregation, platelet production, antiplatelet therapy, coronary artery disease

Introduction
Coronary artery disease (CAD) is a major cause of deaths worldwide and its prevalence is increasing (1). Growing evidence supports that inflammation is involved in all stages of coronary atherosclerosis from fatty streaks and plaque formation to plaque rupture leading to acute coronary syndromes (ACS) (2). Atherosclerosis is characterized by a low-grade, inflammatory process (2). Low-grade inflammation is not clearly defined. Usually, it refers to a subclinical systemic condition determined by increased biomarkers without clinical signs of inflammation. There is no consensus regarding which inflammatory biomarkers or cut-off levels are included in the term. Although the causes of this low-grade inflammatory response remain unknown, inflammation likely reflects the accumulated burden of cardiovascular risk factors such as obesity, diabetes, smoking, and hyperlipidaemia. In this review, we provide a comprehensive overview of inflammation in CAD with particular emphasis on platelet production, activation, and aggregation in CAD patients.

Inflammatory biomarkers in stable coronary disease
Inflammatory biomarkers may provide information on plaque vulnerability and carry the potential to improve risk stratification in CAD patients (3). Furthermore, inflammatory biomarkers may be used as therapeutic targets, counteracting the initiation and progression of atherosclerosis and atherothrombotic events (4). Currently studied inflammatory biomarkers include acute-phase reactants, cytokines, cellular adhesion molecules, markers of plaque destabilisation and rupture, and markers of lymphocyte and monocyte activation (Table 1). Even subtle increases in inflammatory biomarkers have been reported to be associated with

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Table 1: Inflammatory biomarkers and their role in coronary artery disease.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Place of production and effect</th>
<th>Role in coronary artery disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute-phase reactants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP&lt;br&gt;(11, 12, 14, 15, 112, 136)</td>
<td>Produced by hepatocytes, adipocytes, and monocytes/macrophages in response to pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α. Acute-phase protein binding to foreign and damaged cells to facilitate phagocytosis by macrophages.</td>
<td>Contributes to plaque formation by stimulating vascular smooth muscle migration, proliferation, and neoointimal formation. Contributes to plaque destabilisation by stimulating matrix degradation. Associated with increased platelet aggregation in stable CAD patients.</td>
</tr>
<tr>
<td>Fibrinogen&lt;br&gt;(25–28)</td>
<td>Produced by hepatocytes in response to IL-1, IL-6, and TNF-α. The final step in the coagulation cascade as the substrate for thrombin. Inter-platelet bridging in thrombus formation through binding to glycoprotein IIb/IIIa receptors on platelet membranes.</td>
<td>Elevated fibrinogen levels are associated with the formation of compact fibrin clot networks causing resistance to fibrinolysis in patients with CAD and type 2 diabetes. Nearly two-fold risk of thrombotic cardiovascular events in CAD patients with high fibrinogen levels.</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDF-1, CXCL12&lt;br&gt;(44, 46, 47)</td>
<td>Small cytokine secreted in response to vascular injury or ischaemia. Key role in recruitment of haematopoietic progenitor cells for tissue regeneration and neovascularisation.</td>
<td>Cardioprotective effect after myocardial infarction. Increased levels are associated with improvement of left ventricular ejection fraction after MI. Enhanced platelet SDF-1 levels in ACS patients compared with stable CAD patients.</td>
</tr>
<tr>
<td>CXCL16&lt;br&gt;(50, 53, 89, 90)</td>
<td>Chemokine containing transmembrane domain enabling cell adhesion. Produced by several inflammatory cells preferentially within atherosclerotic plaques.</td>
<td>Surface expressed on platelets and released upon platelet stimulation. May also trigger platelet activation and adhesion. Enhanced levels in platelets from ACS patients compared with stable CAD patients. Predicts long-term mortality in ACS patients.</td>
</tr>
<tr>
<td>MIF&lt;br&gt;(54–60)</td>
<td>Chemokine secreted in response to inflammatory stimuli. Important role in monocyte recruitment and arrest through binding to the chemokine receptors CXCR2 and CXCR4.</td>
<td>Involved in plaque vulnerability, progression and rupture by regulating monocyte recruitment towards atherosclerotic lesions. Increased levels in ACS patients compared with stable CAD patients. The prognostic role of MIF in CAD is controversial.</td>
</tr>
<tr>
<td>RANTES&lt;br&gt;(63, 65–69, 126)</td>
<td>Chemokine secreted from platelets following platelet activation. Deposited on inflamed or atherosclerotic endothelium with subsequent recruitment of monocytes onto the activated endothelium.</td>
<td>High RANTES plaque levels have been associated with an unstable plaque phenotype. Transiently increased during episodes of UAP. Results regarding the predictive value in CAD are conflicting.</td>
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<td><strong>Markers of leukocyte activation</strong></td>
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<tr>
<td>Calprotectin&lt;br&gt;(71, 74–79)</td>
<td>Inflammation-associated protein mainly expressed by and released from myeloid cells upon cellular activation.</td>
<td>Early marker to discriminate between patients with ACS and patients with stable CAD. Associated with increased platelet aggregation and thromboxane-dependent activation in CAD patients. Elevated levels have been associated with increased risk of first and recurrent cardiovascular events.</td>
</tr>
<tr>
<td>MPO&lt;br&gt;(138–140)</td>
<td>Secreted from neutrophils at sites of inflammation.</td>
<td>Correlates with degree of endothelial dysfunction. Involved in plaque progression and vulnerability. Elevated levels in stable CAD and ACS. Independent predictor of short-term adverse cardiac events in ACS patients.</td>
</tr>
<tr>
<td>MMPs&lt;br&gt;(141–143)</td>
<td>Expressed in monocytes/macrophages, endothelial cells, smooth muscle cells, fibroblasts, and neoplastic cells. Collectively MMPs have the ability to completely degrade collagen and other components of the extracellular matrix.</td>
<td>Involved in vascular and cardiac post-MI remodelling, including atherogenesis and plaque destabilisation. MMP-2 and MMP-9 are higher in ACS patients than in stable CAD patients.</td>
</tr>
<tr>
<td>Lp-PLA2&lt;br&gt;(144–148)</td>
<td>Produced by macrophages, T lymphocytes, and mast cells.</td>
<td>Elevated levels associated with endothelial dysfunction, carotid atherosclerosis, and increased arterial stiffness in stable CAD patients. Suggested as independent predictor of adverse cardiovascular events in healthy individuals and in stable CAD patients.</td>
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<tr>
<td><strong>Other markers</strong></td>
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<tr>
<td>Copeptin&lt;br&gt;(80, 83)</td>
<td>Secreted from the neurohypophysis in response to haemodynamic or osmotic stimuli.</td>
<td>Associated with adverse cardiovascular events in CAD patients.</td>
</tr>
</tbody>
</table>

*Including neutrophils, monocytes, macrophages, lymphocytes, myelocytes, and non-leukocyte cells, see text in table for further specification. ACS: Acute coronary syndrome; MI: Myocardial infarction; CAD: Coronary artery disease; CRP: C-reactive protein; CXCL16: CXC motif ligand 16; IL: Interleukin; Lp-PLA2 Lipoprotein-Associated Phospholipase A2; MIF: Macrophage migration inhibitory factor; MMP: Matrix metalloproteinases; PDI: Myeloperoxidase; mRNA: messenger ribonucleic acid; SDF-1: stromal-cell derived factor-1; Th cells: T-helper cells; TNF: Tumour necrosis factor; RANTES: Regulated on activation, normal T cell expressed and secreted; UAP: Unstable angina pectoris.
Coronary atherosclerosis is the primary cause of coronary thrombosis (5). The ultimate complication of coronary atherosclerosis is rupture of an atherosclerotic plaque causing exposure of the thrombogenic lipid-core. This promotes platelet adhesion to von Willebrand factor and subendothelial collagen via glycoprotein receptors. Once exposed to local agonists, including adenosine diphosphate (ADP), serotonin, thromboxane A2, thrombin, and collagen, platelet activation and degranulation follows (5). As a part of degranulation, the α-granules of activated platelets release a large number of inflammatory chemokines, cytokines, and growth factors (Figure 1). These substances promote interactions between platelets and endothelial cells as well as leukocytes and endothelial cells. Moreover, they induce endothelial inflammation and release of pro-inflammatory cytokines, which are central in thrombus formation (2).

Chronic low-grade inflammation may aggravate atherogenesis not only by directly promoting the formation of atherosclerotic plaques, but also indirectly by inducing a more atherogenic profile (6). In patients with stable CAD, several inflammatory biomarkers have been investigated to improve our understanding of atherogenesis and improve risk stratification (7). Below, we discuss important inflammatory biomarkers reported to be associated with increased risk of cardiovascular events in patients with CAD.

C-reactive protein

C-reactive protein (CRP) is an acute-phase protein produced by hepatocytes and adipocytes in response to interleukin (IL)-1, IL-6, and tumour necrosis factor (TNF)-α stimulation. CRP has been suggested to induce a prothrombotic state, possibly by enhancing procoagulant activities or by inhibiting fibrinolysis (8).

CRP is the most extensively studied inflammatory biomarker in patients with CAD. In a recent meta-analysis, the cardiovascular risk associated with one standard deviation increase in high-sensitivity (hs)CRP is at least as high as the risk associated with one standard deviation increase in either total cholesterol or blood pressure (9). Thus, in contrast to many other novel biomarkers, hsCRP adds...
to traditional risk factors included in risk scores for cardiovascular risk prediction (10).

Elevated CRP levels have been associated with increased risk of cardiovascular events in patients with stable CAD (11–13) as well as ACS (14, 15). Previous studies have shown that high hsCRP levels for at least three months following an acute ischaemic event are associated with recurrent events (14, 16). Therefore, it is clinically relevant to identify patients with a prolonged inflammatory response in order to improve risk stratification. In a large study of stable CAD patients, one standard deviation increase in hsCRP was associated with a 50% increase in the risk of coronary events (17).

In some studies, CRP levels have been proposed to reflect the extent of underlying atherosclerosis (18, 19), whereas other studies did not find such an association (16, 20). Cheng et al. reported that elevated levels of hsCRP were independently associated with higher coronary plaque burden (21) and Kubo et al. found that the percentage of necrotic plaque core was independently associated with elevated hsCRP levels (22).

In summary, across large prospective studies, hsCRP has been consistently documented as a risk factor integrating multiple metabolic and low-grade inflammatory factors underlying the development of unstable atherosclerotic plaques with an ability to predict cardiovascular events matching that of classical clinical risk factors. However, several issues complicate the inclusion of this biomarker as a tool for risk assessment: lack of precision with a narrow diagnostic window for hsCRP levels and risk of cardiovascular disease; lack of specificity with similar levels of risk for non-cardiovascular causes of morbidity and mortality (e.g. other low-grade inflammatory diseases); and finally, lack of causality through randomised controlled trials to prove a dose-effect of reduced hsCRP levels and risk of cardiovascular disease. Current guidelines from the European Society of Cardiology do not recommend routine measurement of hsCRP in risk stratification used for patients with stable CAD (23).

Fibrinogen

Fibrinogen is an acute-phase reactant. It represents the final step in the coagulation cascade being substrate for thrombin and, moreover, it is essential for platelet aggregation through binding to glycoprotein IIB/IIIa receptors on the platelet membrane (24). Cytokines including IL-6, IL-1, and TNF-α may induce production of fibrinogen by the liver resulting in increasing plasma levels of fibrinogen (24). In population-based studies, increased plasma levels of fibrinogen have been associated with increased risk of thrombotic events (25).

A meta-analysis by Danesh et al. reported a nearly two-fold increased risk of thrombotic cardiovascular events in the upper versus the lower tertile of fibrinogen levels in CAD patients (26). In CAD patients with type 2 diabetes, increased fibrinogen levels have been associated with increased platelet aggregation (25, 27) as well as formation of more compact fibrin clot networks causing resistance to fibrinolysis (28). A recent randomised trial testing fibrinogen-lowering treatment with bezafibrate did not show a reduction in cardiovascular risk (29). It remains to be clarified whether the association between fibrinogen and cardiovascular risk is a cause, consequence or a mere coincidence.

Interleukin-6

IL-6 is the major initiator of acute-phase responses and the primary determinant of hepatic CRP production (30). Experimental studies indicate that vascular endothelial and smooth muscle cells produce IL-6 (31) and that IL-6 gene transcripts are expressed in human atherosclerotic lesions (32). Elevated IL-6 levels have been associated with increased cardiovascular risk in healthy men (33) and women (34), and in elderly patients (35).

IL-6 is an important player in the development and progression of atherosclerosis (36). Increased IL-6 levels have been demonstrated in patients with ACS (37–40), but not in patients with stable angina (37, 40). The prognostic potential of IL-6 in the context of pre-existing cardiovascular disease has not been fully clarified (33, 40). In patients with stable CAD, elevated baseline IL-6 levels were associated with recurrent ischaemic events (41, 42). The discovery that cytokine production is elevated not only in ACS patients, but also in patients with stable CAD, may reflect prolonged duration of inflammatory processes in the vascular wall.

Stromal cell-derived factor-1

Stromal cell-derived factor-1 (SDF-1; CXCL12) is a small cytokine expressed by several cells and stored in platelet α-granules and secreted at sites of vascular injury or ischaemia (43). SDF-1 plays a crucial role in trafficking haematopoietic progenitor cells for tissue regeneration and neovascularisation via its cognate receptors CXCR4 and CXCR7, and it may be cardioprotective in myocardial infarction (44, 45).

Platelet SDF-1 surface expression is significantly increased in ACS patients compared with stable CAD patients (44, 46). Furthermore, in patients with previous myocardial infarction, enhanced platelet SDF-1 expression was associated with reduced infarct size and significant improvement of left ventricular ejection fraction (47). In a recent study, lower levels of the platelet SDF-1 cognate receptor CXCR4 were shown to be independently associated with all-cause mortality and a combined end-point of all-cause mortality and/or myocardial infarction, suggesting a potential prognostic value of CXCR4 platelet expression in CAD patients (48).

CXC motif ligand 16

CXC motif ligand (CXCL)16 is a newly discovered molecule, which combines functions of a chemokine, a scavenger receptor, and an adhesion molecule (49). Along with CX3CL1, CXCL16 is the only chemokine that enables cell adhesion because it contains a transmembrane domain. CXCL16 is produced by several inflammatory cells and preferentially expressed within atherosclerotic plaques (50).
CXCL16 is associated with inflammation and progression of CAD (51, 52). Furthermore, enhanced levels of CXCL16 have been associated with long-term mortality in ACS patients (53).

**Macrophage migration inhibitory factor**

Macrophage migration inhibitory factor (MIF) is a chemokine secreted in response to inflammatory stimuli (54). It plays an important role in monocyte recruitment through binding to the chemokine receptors CXCR2 and CXCR4, and is involved in plaque progression and rupture by regulating monocyte recruitment towards atherosclerotic lesions (55).

Circulating levels of MIF have been shown to reflect severity of chronic inflammatory diseases including CAD (56, 57), and MIF levels are significantly increased in ACS patients compared with stable CAD patients (58, 59). However, the predictive value of MIF for future cardiac events remains controversial (56, 57, 60).

**RANTES**

RANTES (regulated on activation, normal T cell expressed and secreted) is a platelet chemokine stored in α-granules, which can be rapidly released following platelet activation (61). RANTES can be identified on the luminal surface of atherosclerotic murine and human carotid arteries (62, 63) or in neointimal lesions after arterial injury and can be deposited on inflamed endothelium by activated platelets thereby triggering monocyte recruitment (63, 64).

Data regarding the significance of plasma RANTES levels in CAD patients are conflicting. On the one hand, levels of RANTES have been reported to be elevated in ACS patients (65), whereas levels are down regulated in stable CAD patients (66). In ACS patients, elevated levels of RANTES were independently associated with risk of short-term mortality (65, 67), however, another study showed no association between RANTES and adverse outcome (68). Furthermore, some studies even reported low levels of RANTES to be associated with disease progression and to be an independent predictor of adverse events (66, 69). Additionally, gene polymorphisms expected to result in reduced levels of RANTES have been associated with adverse cardiac events (70), and it has been speculated that RANTES may have divergent implications in stable versus unstable CAD patients.

**Calprotectin**

Calprotectin, also known as myeloid-related protein 8/14, S100A8/A9 or calgranulin A/B, is an inflammation-associated protein mainly expressed by and released from myeloid cells upon cellular activation (71). Traditionally, calprotectin plasma levels have primarily been investigated as a marker of inflammatory conditions such as inflammatory bowel disease and rheumatoid arthritis (72, 73). However, recent studies suggest that calprotectin may be implicated in the pathogenesis of CAD (74–76) and has also been identified as a sensitive biomarker enabling the discrimination between ACS patients and patients with stable CAD (76).

Calprotectin has been associated with increased risk of first and recurrent cardiovascular events (74, 75, 77). Recently, our group demonstrated a positive correlation between high calprotectin levels and increased platelet aggregation in stable, high-risk CAD patients (78). Furthermore, calprotectin was positively associated with serum thromboxane B2, hsCRP, and IL-6 (78). In ACS patients, Santilli et al. reported an association between circulating calprotectin with thromboxane-dependent platelet activation even during low-dose aspirin treatment, suggesting a contribution of residual thromboxane to calprotectin shedding, which may further amplify platelet activation (79).

**Copeptin**

Copeptin is the precursor peptide of vasopressin and is secreted from the neurohypophysis (80). It may increase the diagnostic capacity of troponin when the two markers are evaluated in combination in the early diagnosis of acute myocardial infarction (81).

Copeptin has been suggested as an independent prognostic marker in patients with heart failure following acute myocardial infarction (82). Recently, the prognostic value of copeptin was evaluated in a large CAD population presenting with chest pain, and elevated levels of copeptin were significantly associated with a higher rate of major adverse cardiovascular events (83). Thus, copeptin may be useful as a diagnostic marker in the early phase of acute myocardial infarction as well as a predictive marker for major adverse cardiovascular events in CAD patients.

**Other inflammatory biomarkers**

Numerous additional inflammatory markers including, but not limited to, matrix metalloproteinases, myeloperoxidase, lipoprotein-associated phospholipase/fractalkine may be implicated in atherosclerosis and have been suggested as potential predictors of adverse events in CAD patients (Table 1 and Table 2).

**Impact of inflammation on platelet function**

In addition to their central role in haemostasis, platelets are essential in inducing and maintaining inflammation. Activated platelets interact with several different cell types present in the arterial wall (e.g. monocytes, neutrophils, endothelial cells, and endothelial progenitor cells) by direct receptor interactions, but also through autocrine and paracrine pathways (84). Platelets contain several intracellular compartments, including three different granules (α-granules, lysosomes, and dense core granules), and a complex membranous system allowing the storage and rapid release of a variety of factors (Figure 1).

Chemokines secreted by platelets and other cells stimulate platelet activation and adhesion with subsequent amplification of the activation-dependent release of proatherogenic and prothrombotic proteins from platelet granula (84). Below, we will focus on the impact of SDF-1, CXCL16 and RANTES.
**Stromal-cell derived factor-1**

When activated by collagen or by endothelial adhesion, human platelets release and present SDF-1 on their surface. SDF-1 activates its cognate receptor CXCR4 on CD34+ progenitor cells, which leads to adhesion and endothelial differentiation (45). SDF-1 has been suggested as a potent chemokine directly inducing platelet activation in healthy individuals (85), a process that can be inhibited by blocking CXCR4, implying an atherogenic, prothrombotic and plaque-destabilising role for the SDF-1/CXCR4 axis in vivo (88). On the contrary, other studies have concluded that SDF-1 is a rather weak platelet agonist, although still able of amplifying platelet activation, adhesion and chemokine release triggered by low doses of ADP and thrombin (86, 87). A recent study suggested that SDF-1 may also be involved in regulation of platelet turnover as SDF-1 was able to trigger CXCR4 internalisation and cyclophilin A-dependent CXCR7 externalisation in both mouse and human platelets resulting in prolonged platelet survival (88).

**CXCL16**

CXCL16 has been shown to be surface-expressed on platelets and is released upon platelet stimulation (89). Furthermore, a recent study reported that CXCL16 triggers platelet activation and adhesion and suggested a decisive role for CXCL16 in linking vascular inflammation and thrombo-occlusive diseases (90).

**RANTES**

In apolipoprotein E-"- mice injected with activated platelets, immobilisation of RANTES by platelets seems to be involved in atherogenesis (91). Conversely, injection of P-selectin-deficient platelets, which are incapable of depositing RANTES, did not induce native atherosclerosis (91). Blocking RANTES receptors in a hyperlipidaemic mouse model have been associated with reduced atherosclerosis not only by preventing monocyte recruitment to the vessel wall, but also by limiting the circulating inflammatory monocytes (62, 92), supporting the role of RANTES in vivo.

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### Table 2: Overview of clinical studies investigating predictive properties of inflammatory biomarkers in patients with coronary artery disease.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Author</th>
<th>Study design</th>
<th>n</th>
<th>Follow-up</th>
<th>End point; RR, HR or OR (95% CI), p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute-phase reactants</strong></td>
<td></td>
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<tr>
<td>CRP</td>
<td>Haverkate et al. (17)</td>
<td>Observational</td>
<td>2121</td>
<td>24 months</td>
<td>Coronary events; 1.45 (1.5–1.83), p=0.002</td>
</tr>
<tr>
<td></td>
<td>Bogaty et al. (149)</td>
<td>Observational</td>
<td>1210</td>
<td>12 months</td>
<td>Death, nonfatal MI, UAP; 1.12 (0.93–1.34), p=0.24</td>
</tr>
<tr>
<td></td>
<td>Sabatine et al. (12)</td>
<td>Substudy of RCT</td>
<td>3771</td>
<td>4.8 years</td>
<td>CV death, MI, stroke; 1.52 (1.15–2.02), p=0.003</td>
</tr>
<tr>
<td></td>
<td>He et al. (150)</td>
<td>Meta-analysis</td>
<td>9877</td>
<td>1–96 months</td>
<td>Death, CV events; 2.18 (1.77–2.68), p&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Danesh et al. (26)</td>
<td>Meta-analysis</td>
<td>4018</td>
<td>8 years</td>
<td>Coronary heart disease; 1.8 (1.6–2.0)*</td>
</tr>
<tr>
<td></td>
<td>Van Loon et al. (151)</td>
<td>Observational</td>
<td>353</td>
<td>4.2 years</td>
<td>All-cause mortality; 1.79 (0.80–3.97)*</td>
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<tr>
<td><strong>Cytokines</strong></td>
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<tr>
<td>IL-6</td>
<td>Lindmark et al. (137)</td>
<td>RCT</td>
<td>2257</td>
<td>12 months</td>
<td>Death; 2.08 (1.24–3.49), p=0.006</td>
</tr>
<tr>
<td>SDF-1, CXCL12 (CXCR4)</td>
<td>Rath et al. (48)</td>
<td>Observational</td>
<td>284</td>
<td>12 months</td>
<td>All-cause death, MI; 0.30 (0.13;0.72)*</td>
</tr>
<tr>
<td>CXCL16</td>
<td>Jansson et al. (53)</td>
<td>Observational</td>
<td>1351</td>
<td>81 months</td>
<td>All-cause death; 2.1 (1.6–2.8), p&lt;0.0001</td>
</tr>
<tr>
<td>MIF</td>
<td>Makino et al. (60)</td>
<td>Observational</td>
<td>617</td>
<td>50 months</td>
<td>Cardiac death, MI, UAP; 3.3 (1.6–8.3), p=0.006</td>
</tr>
<tr>
<td>RANTES</td>
<td>Herder et al. (68)</td>
<td>Case-cohort</td>
<td>363 / 1908</td>
<td>10.2 years</td>
<td>CV death, MI; 1.11 (0.81–1.54), p=0.53</td>
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<tr>
<td><strong>Markers of leukocyte activation</strong></td>
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<tr>
<td>Calprotectin</td>
<td>Morrow et al. (74)</td>
<td>Case-control</td>
<td>237</td>
<td>30 days</td>
<td>CV death, MI; 2.0 (1.1–3.6), p=0.029</td>
</tr>
<tr>
<td></td>
<td>Jensen et al. (152)</td>
<td>Observational</td>
<td>141</td>
<td>12 months</td>
<td>Mortality; 11.11 (2.2–56.0), p=0.004</td>
</tr>
<tr>
<td>MPO</td>
<td>Morrow et al. (140)</td>
<td>Observational</td>
<td>1524</td>
<td>30 days</td>
<td>Non-fatal recurrent ischaemic events; 2.10 (1.36–3.23), p=0.001</td>
</tr>
<tr>
<td>Lp-PLA2</td>
<td>Sabatine et al. (146)</td>
<td>Observational</td>
<td>3766</td>
<td>4.8 years</td>
<td>CV death, MI, revascularisation, UAP, stroke; 1.41 (1.17–1.70), p&lt;0.001</td>
</tr>
<tr>
<td><strong>Other markers</strong></td>
<td>Voors et al. [82]</td>
<td>Observational</td>
<td>224</td>
<td>33 months</td>
<td>Mortality; 1.83 (1.26–2.64), p&lt;0.0001</td>
</tr>
</tbody>
</table>

*P-values not provided for some analysis. MI: Myocardial infarction; CI: confidence interval; CRP: C-reactive protein; CV: cardiovascular; CXCL16: CXC motif ligand 16; IL: Interleukin; Lp-PLA2: Lipoprotein-Associated Phospholipase A2; MIF: Macrophage migration inhibitory factor; OR: odds ratio; SDF-1: stromal-cell derived factor-1; RANTES: regulated on activation, normal T cell expressed and secreted; RCT: randomised controlled trial; RR: relative risk; UAP: Unstable angina pectoris.
Inflammatory markers and platelet production

Changes in megakaryocytopoiesis and platelet production may contribute to development of thrombosis. Conversely, atherosclerosis may modify megakaryocyte and platelet biology, possibly through inflammatory mediators. Importantly, the regulation of platelet production and thrombopoietin has been studied primarily in experimental models or in patients with haematological disorders (93), which are very different settings than CAD.

Newly formed platelets, referred to as reticulated or immature platelets, are larger and have a higher number of dense granules compared with mature platelets (94). Recently, our group found that thrombopoietin was an independent predictor of immature platelet fraction and immature platelet count (95). However, only a few studies have investigated the association between thrombopoietin and platelet turnover parameters in CAD patients (96–98) and, so far, no firm conclusions can be drawn.

Even though thrombopoietin is considered the key hormone in the regulation of platelet production, it likely acts in conjunction with numerous cytokines (93). In experimental studies, IL-6 has been shown to promote megakaryocyte differentiation (99, 100), and infusion of IL-6 has been shown to induce modest thrombocytosis (101). Therefore, inflammation associated with atherosclerotic disease may impact platelet formation from megakaryocytes directly through IL-6-dependent mechanisms (102).

In a large population of stable CAD patients, our group recently reported that high hsCRP levels correlated positively with increased platelet count, immature platelet count, and thrombopoietin levels (96). In the same study, we found that IL-6 correlated with thrombopoietin, but otherwise there were no associations between platelet turnover parameters and low-grade inflammatory markers (96). In another study, increased hsCRP levels were independently related with mean platelet volume suggesting that high mean platelet volume may be part of the chronic low-grade inflammatory state in stable CAD patients (103). Lupia et al. found significantly higher levels of thrombopoietin and CRP in patients with unstable angina as compared with patients having stable angina and suggested that the acute-phase response related to ACS may increase thrombopoietin levels (97).

Inflammation and antiplatelet therapy

Aspirin

Low-dose aspirin given once daily is a mainstay in secondary prevention of CAD and reduces serious cardiovascular events by approximately 25% (104). Aspirin inhibits platelet aggregation by irreversibly acetylating the cyclooxygenase-1 enzyme, thereby inhibiting the conversion of arachidonic acid to thromboxane A2 (105). However, CAD patients display considerable variability in the effect of aspirin, most likely as a consequence of genetic, biological, and clinical factors (106, 107).

Chronic low-grade inflammation has been proposed to modify platelet aggregation leading to a reduced antiplatelet effect of aspirin in CAD patients (108–114). Our group recently found that hsCRP independently predicted increased arachidonic acid-induced platelet aggregation, whereas IL-6 did not (112). These results indicate an association between chronic low-grade inflammation and reduced antiplatelet effect of aspirin, which is consistent with a number of smaller studies (108, 109, 113). Previous studies have shown associations that may partly explain the interaction between inflammation and antiplatelet drug efficacy. CRP has been shown to directly affect monocytes via the FcγRIIa receptor, an activation which may subsequently affect platelets (115). Furthermore, CRP induction of macrophage-derived tissue factor generation has been proposed as a potential mechanism explaining increased platelet aggregation and chemokine secretion (116). Nonetheless, a direct interaction between CRP and platelet aggregation has not been established.

High platelet aggregation levels have also been found to be associated with significantly increased levels of von Willebrand factor, platelet count, total tissue factor pathway inhibitor, and β-thromboglobulin (114). The authors suggested that elevated levels of these markers in patients with high on-aspirin platelet aggregation may be explained by increased endothelial cell activation and platelet activation. Also in stable CAD patients, Karolczak et al. reported an association between CRP, LDL-cholesterol, and reduced antiplatelet effect of aspirin (111), and others reported that increased levels of hsCRP and IL-6 were independently associated with increased platelet aggregation and urine-11-dehydro-thromboxane B2 levels (110). This association may be explained by aspirin-insensitive thromboxane generation derived from cyclooxygenase-2 in non-platelet cells.

In patients with ACS, Gori et al. investigated the association between several inflammatory markers and platelet aggregation, but only found an association with interferon-gamma and IL-4 (117). A positive correlation between soluble CD40 ligand, CRP, and IL-8 and thrombogenicity has been demonstrated in patients undergoing coronary angioplasty (118, 119). Others reported that soluble CD40 ligand may initiate platelet aggregation and is correlated with plasma P-selectin and urinary thromboxane levels (120, 121). Conversely, in aspirin-treated patients with stable CAD, other studies did not show a positive association between hsCRP or soluble CD40 ligand levels and platelet aggregation levels (122).

In summary, low-grade inflammation may have an impact on the antiplatelet effect of aspirin in stable CAD patients. However, the existing studies were not designed to prove causality.

Clopidogrel

Clopidogrel is a second-generation thienopyridine and a prodrug that requires hepatic bioactivation involving a two-step oxidative process regulated by the cytochrome P450 (CYP) system. Interestingly, the hepatic CYP system, including CYP3A4, is down-regulated in hepatocytes in response to inflammation (123). By this mechanism, inflammation may have critical impact on the response to drugs metabolised in the liver.

A limited number of studies have addressed the relationship between inflammation and the antiplatelet effect of clopidogrel.
Bernlochner et al. explored the association between inflammatory biomarkers and platelet aggregation in stable CAD patients treated with aspirin and clopidogrel undergoing percutaneous coronary intervention (124). In an analysis adjusting for known predictors of high on-clopidogrel platelet aggregation, high levels of CRP, fibrinogen, and leukocytes were independently associated with high residual ADP-induced platelet aggregation. The association was robust across all biomarkers thus likely being driven by inflammation per se rather than being specific for a single biomarker (124). The reported association between high CRP levels and residual ADP-induced platelet aggregation has been supported by smaller cohort studies (125, 126).

In patients with diabetes, Ang et al. demonstrated that high fibrinogen levels were associated with high platelet aggregation (127). On the contrary, according to Gaborit et al., fibrinogen does not directly affect clopidogrel response, but more likely represents a systemic inflammatory response that may be associated with increased platelet aggregation (128). They showed that addition of large amounts of purified fibrinogen to platelet-rich plasma did not modify ADP-induced platelet aggregation in patients already categorised as clopidogrel non-responders. Moreover, the study showed that fibrinogen levels were associated with ADP-induced aggregation, but not with the platelet reactivity vasodilator-stimulated phosphoprotein (VASP)-index. This argues against the above-mentioned hypothesis that inflammatory markers reduce the effect of clopidogrel by down-regulating clopidogrel metabolism. Accordingly, ADP-induced aggregation reflects platelet function more generally than the VASP-index, which specifically reflects intracellular P2Y12 pathway inhibition.

As pathways of thrombosis and inflammation are interlinked, clopidogrel may also possess anti-inflammatory effects. For instance, clopidogrel seems to inhibit platelet-leukocyte conjugate formation (129) and platelet-dependent leukocyte activation (130). Supporting such pleiotropic effects of clopidogrel, withdrawal of clopidogrel is associated not only with pro-thrombotic effects, but also pro-inflammatory effects (131). Gori et al. reported an inverse relation between IL-4 and IL-10 and platelet reactivity in aspirin- and clopidogrel-treated ACS patients (132), whereas others found that IL-10 levels were increased in clopidogrel non-responders (133), a discrepancy that may reflect that the distinction between pro- and anti-inflammatory mediators is complex and poorly defined.

Theoretically, a connection between inflammation and the antiplatelet effect of clopidogrel is plausible, yet available evidence is based on association studies, from which a causal relation cannot be inferred.

**Anti-inflammatory drugs for coronary artery disease**

Emerging anti-inflammatory drugs for the management of CAD can be categorised into two groups: those that target the IL-6 signalling pathway (such as methotrexate and canakinumab) and those that do not (e.g. darapladib and varespladib). In 2013, the Cardiovascular Inflammation Reduction Trial (CIRT) was initiated (134). In this ongoing trial, 7,000 patients with chronic atherosclerosis and either diabetes mellitus or metabolic syndrome are randomised to usual care plus placebo or low-dose methotrexate (15–20 mg per week). The primary endpoint is recurrent non-fatal cardiovascular events and cardiovascular mortality. The ongoing Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) trial enrolls 10,000 patients with stable CAD who have persistent elevation of CRP (>2 mg/l) to investigate whether canakinumab reduces the recurrence of major cardiovascular events (135). Recently, the Controlled Level EVERolimus in Acute Coronary Syndromes (CLEVER-ACS) trial (ClinicalTrials.gov Identifier: NCT01529554) started enrolling patients with ST-segment elevation myocardial infarction to determine whether anti-inflammatory therapy with everolimus will reduce infarct size, remodelling, and clinical events.

**Conclusion**

Substantial experimental and clinical evidence suggests that inflammation plays a crucial role in the initiation and progression of atherosclerosis. Numerous biomarkers involved at various levels of the inflammation cascade have been associated with cardiovascular outcomes. Low-grade inflammation may influence both platelet production and the effect of antiplatelet drugs in patients with stable CAD. Yet, fundamental gaps of knowledge remain, since existing data do no clarify whether inflammation simply accompanies CAD and increased platelet production and platelet aggregation, or whether a causal relationship exists.

**Conflict of interests**

The authors report the following general conflicts of interest: SDK has received speaker honoraria from Aspen, AstraZeneca and The Medicines Company. ELG has received speaker honoraria from AstraZeneca, Baxter, Bayer, Boehringer Ingelheim, Pfizer and Symex, and has participated in advisory board meetings for AstraZeneca, Bayer, and Bristol-Myers Squibb. AMH has received speaker’s fees from CSL Behring and Leo Pharma and research support from Octapharma, CSL Behring and Leo Pharma. MW has received financial support for scientific purposes from Bristol-Myers Squibb. SBL and SNP have no conflicts of interest to declare.

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