Chemokines and Cardiovascular Risk

Pål Aukrust, Bente Halvorsen, Arne Yndestad, Thor Ueland, Erik Øie, Kari Otterdal, Lars Gullestad, Jan K. Damås

Abstract—Based on the importance of inflammation in atherogenesis, recent work has focused on whether plasma markers of inflammation can noninvasively diagnose and prognosticate atherosclerotic disorders. Although several studies support an important pathogenic role of chemokines in atherosclerosis, potentially representing attractive therapeutic targets in atherosclerotic disorders, this does not necessarily mean that chemokines are suitable parameters for risk prediction. In fact, the ability to reflect upstream inflammatory activity, stable levels in individuals, and high stability of the actual protein (eg, long half-life and negligible circadian variation) are additional important criteria for an ideal biomarker in cardiovascular disease. Although plasma/serum levels of certain chemokines (eg, interleukin-8/CXCL8 and monocyte chemoattractant protein-1/CCL2) have shown to be predictive for future cardiac events in some studies, their role as clinical biomarkers is unclear, and their ability to predict subclinical atherosclerosis has been disappointing. Further prospective studies, including a larger number of patients, are needed to make any firm conclusion. Based on the participation of several chemokines in atherogenesis, it is possible that in the future, combined measurements of multiple chemokines could reveal as a “signature of disease” that can serve as a highly accurate method to assess for the presence of atherosclerotic disease. (Arterioscler Thromb Vasc Biol. 2008;28:1909-1919)

Key Words: chemokines ■ atherosclerosis ■ biomarkers ■ inflammation

A decade ago, lipid lowering therapy was expected to eliminate coronary artery disease (CAD) and other forms of atherosclerosis by the end of the 20th century. However, that optimistic prediction has needed revision and cardiovascular diseases are expected to be the main cause of death globally within the next 15 years, at least partly owing to a rapidly increasing prevalence in developing countries and Eastern Europe and the rising incidence of obesity and diabetes in the Western world.1 At present, cardiovascular diseases cause 38% of all deaths in North America and are the most common cause of death in European men under 65 years of age and the second most common cause in women. These facts force us to revisit cardiovascular disease and consider new strategies for prevention and treatment as well as for disease and risk prediction. Commonly used risk algorithms, such as the Framingham Risk Score, and lipid parameters fail to identify all affected individuals.1,2 Novel cardiovascular risk factors that identify these missed individuals would greatly improve overall care of patients. These risk markers should also give prognostic information in patients with established cardiovascular disease as well as being predictors of efficacy for various therapeutic interventions (eg, statins and different forms of antithrombotic medications).1,2 Ideally, new risk markers should also reflect important pathogenic processes in atherogenesis and plaque destabilization, and the attempt to identify new biomarkers in atherosclerotic disorders is therefore linked to the study of the pathogenic mechanisms of atherosclerosis.

The Role of Chemokines in Atherogenesis

Atherosclerosis is a chronic disease characterized by two fundamental hallmarks: lipid accumulation and inflammation.1-3 The interaction between these two processes defines the principal pathogenesis and distinguishes atherosclerosis from other chronic inflammatory disorders. Although the concept that atherosclerosis is an inflammatory disease is no longer controversial, the regulation of these inflammatory processes as well as their pathogenic consequences is not...
fully understood. Moreover, although the participation of inflammation in atherogenesis has become widely recognized, the identification and characterization of the different actors and their relative importance are not fulfilled. However, several lines of evidence suggest that chemokines are important actors in this immune-mediated process, leading to progression of atherosclerosis and plaque destabilization (Figures 1 and 2).

Chemokines are inflammatory cytokines characterized by their ability to cause directed migration of leukocytes into inflamed tissue, and raised levels are found in atherosclerosis, both systemically and within the atherosclerotic plaques. Hence, there are several reports of enhanced expression of CXC-chemokines (eg, IL-8 [IL-8/CXCL8], neutrophil-activating peptide-2 [NAP-2/CXCL7], growth-related oncogene-α [GRO-α/CXCL1], interferon [INF]-γ-inducible 10 [IP-10/CXCL10], and CXCL16), CC-chemokines (eg, monocyte chemoattractant protein-1 [MCP-1/CCL2], leukotactin-1 [Lkn-1/CCL15], regulated on activation, normal T cell expressed and secreted [RANTES/CCL5], CCL19, and CCL21) within human atherosclerotic lesions, and there are also some studies suggesting that these chemokines are colocalized with enhanced expression of their corresponding receptors. The role of chemokines in atherosclerosis is further supported by several studies showing that modified LDL particles are potent inducers of chemokines in various cells such as macrophages and vascular smooth muscle cells (SMC), suggesting that chemokines may represent a link
between lipids and inflammation in atherogenesis.15,16 In addition to being potent chemoattractants, several other leukocyte/macrophage responses such as cell proliferation, enzyme secretion, induction of reactive oxygen species, and promotion of foam cell formation have been observed in vitro after chemokine stimulation.17–19 Moreover, beyond their effects on leukocytes, chemokines may also interfere with SMC migration and growth as well as platelet activation.19–21 Some of these responses may clearly be relevant to atherogenesis, and plaque destabilization, and the importance of chemokines in these processes is supported by several studies in gene-modified mice. Thus, targeted disruption of the genes for CCL2, CCR2 (ie, CCL2 receptor), CXCR2 (ie, CXCL1, CXCL7, and CXCL8 receptor), CXCR6 (ie, CXCL16 receptor), and CX3CR1 (ie, fractalkine/CX3CL1 receptor) significantly decreases atherosclerotic lesion formation and lipid deposition in mice prone to develop atherosclerotic-like lesions.22–27 Furthermore, deletion of CX3CL1 in CCR2+/− ApoE−/− mice dramatically reduces the development of atherosclerosis, providing in vivo evidence for independent roles for CCR2 and CX3CL1 in atherogenesis,28 indicating that successful therapeutic strategies may need to target multiple chemokines or chemokine receptors. This view was supported by Combadière and coworkers showing that combined inhibition of CCL2, CX3CR1, and CCR5 in ApoE-deficient mice leads to an additive reduction in atherosclerosis.29 However, the role of chemokines in atherogenesis is far from completely understood. In fact, studies in gene-modified mice indicate antiatherogenic rather than proatherogenic effects of some of these mediators (eg, deficiency in CXCL16 or CCR1...
accelerates atherosclerosis), suggesting that chemokines also may exert atheroprotective properties, at least when operating at a physiological level. Moreover, studies in gene modified mice should be interpreted with some caution, focusing on atheroclerotic lesion in aorta and not in the coronary circulation. Also, the total lack of one particular chemokine or chemokine receptor may not necessarily give insight into the situation in CAD patients with moderately upregulation of these mediators.

The Role of Chemokines in Plaque Destabilization

Infiltration and activation of leukocytes into the atherosclerotic plaque may also be involved in triggering of acute coronary syndromes (ACS). \(^{1-3}\) Again, chemokines may play an important role in this immune-mediated plaque destabilization, not only by recruiting activated leukocytes into the lesion, but also by directly contributing to plaque rupture and thrombus formation through mechanisms such as enhancement of the matrix degrading potential in macrophages, inducing tissue factor in vascular SMCs and endothelial cells, and by inducing oxidative stress and apoptosis within the atherosclerotic lesion. \(^{18,32,33}\) Moreover, although the role of platelets in thrombus formation is well established, platelet-mediated inflammation could also contribute to the development of ACS. \(^{31,34,35}\) Thus, by releasing a wide range of inflammatory mediators on activation, including several chemokines (eg, CCL5, CXCL1, epithelial neutrophil activating peptide-78 [ENA-78/CXCL5], and CXCL7), platelets may promote inflammatory responses in neighboring leukocytes and endothelial cells, and notably, platelets may themselves respond to inflammatory mediators that are released from these adjacent cells, at least partly through interaction with platelet-expressed chemokine receptors. \(^{21,35}\) Consequently, chemokines could be identified as potential important mediators not only in atherogenesis, but also in plaque destabilization (Figure 2). Such a notion was recently supported in a study by Lutgens et al showing that inhibiting CCL2 and MCP-5/CCL12 induced a stable plaque phenotype in ApoE \(^{-/-}\) mice. \(^{36}\) However, although there are some reports suggesting that ApoE \(^{-/-}\) and LDL receptor (LDLR) \(^{-/-}\) mice may spontaneously develop plaques in certain parts of the arterial tree showing features suggestive of plaque rupture, there is an urgent need for representative animal models where prospective examination of the events leading up to plaque rupture and the rupture process itself can be performed. \(^{37}\)

Inflammatory Mediators as Biomarkers in Cardiovascular Disease

Despite the important role of cholesterol in atherosclerosis, many individuals who experience myocardial infarction (MI) have cholesterol concentrations at or below recommended levels. \(^{1,2}\) Moreover, many patients who present with acute MI are receiving drug therapy for dyslipidemia, despite LDL concentrations at currently mandated targets or below. \(^{38}\) This convergence of clinical findings highlights the necessity of improving our ability to predict cardiovascular risk. In consideration of the important role of inflammation in atherogenesis, recent work has focused on whether plasma markers of inflammation can noninvasively diagnose and prognosticate CAD and other forms of atherosclerosis. \(^{39,40}\) C-reactive protein (CRP), the prototype inflammatory marker, has certainly been the best studied of these “new” biomarkers. As a downstream marker, CRP provides functional integration of upstream cytokine activation, and CRP has proven remarkably robust as a marker of cardiovascular risk and gives predictive value beyond that of traditional risk factors in apparently healthy individuals as well as in CAD patients. \(^{41-45}\) Also, in patients with ACS, CRP levels seem to give prognostic information on mortality. \(^{44}\) Finally, measuring CRP in CAD patients may be useful in monitoring responses to various intervention such as statin therapy. \(^{45}\) However, although CRP is a stable and reliable marker of inflammation and may mirror several aspects of the immunopathogenic mechanisms in CAD, the inflammatory processes that underlie atherosclerosis are mediated by a multitude of cytokines and are unlikely to be reflected by CRP levels alone. \(^{46}\) Thus, CRP seems not to predict early or late occurrence of MI in patients with ACS. \(^{44}\) Moreover, other inflammatory mediators have also been evaluated as biomarkers in CAD, and some of these have been found to give prognostic information beyond that of CRP (eg, IL-6, IL-18, intracellular adhesion molecule [ICAM]-1, osteoprotegerin, and soluble CD40 ligand [sCD40L]). \(^{47-51}\) Finally, although some in vitro studies have claimed that human CRP has proatherogenic effects, the pathogenic role of CRP in atherogenesis is at least controversial. \(^{52}\)

What Is a Reliable Biomarker in Cardiovascular Disease?

A biomarker for cardiovascular disease should reflect important pathophysiological processes in atherogenesis and plaque destabilization, and some biomarkers have failed because they are involved in only one pathway in a multiple-pathway disease or they reflect epiphenomena independent of the disease process. However, although chemokines seem to play a central pathogenic role in atherogenesis, this does not necessarily mean that chemokines are suitable parameters for risk prediction. In fact, the leading role of CRP as an inflammatory biomarker in cardiovascular disease is not primarily based on its pathogenic role in these disorders, but rather on its ability to reflect upstream inflammatory activity. The fact that several different inflammatory markers with different biological activities contribute to the statistical risk for CAD makes it unlikely that CRP or any of the other markers actually causes the disease. Instead, these markers have the ability to reflect the local inflammatory process in the artery and, perhaps, in other tissues and organs with relevance to atherogenesis such as adipose tissue in patients with metabolic syndrome and diabetes mellitus. \(^{7}\) Moreover, a proposed biomarker should not only provide independent information on cardiovascular risk, but also be easy to measure using inexpensive and standardized commercial assays with low variability that do not require specialized plasma collection or assay techniques. In this regard CRP has proved most robust, as it is an excellent analyte with a standardized assay, has negligible diurnal variation, does not
complicated chemokine network, being mediators that are operating rather upstream in the inflammatory cascade.

Chemokines as Markers for Subclinical CAD

Migration of monocytes into the arterial wall is an early event in atheroma formation.1–3 Based on the suggested important role of chemokines in this process, and in particular CXCL8 and CCL2,6,11,23,24 they could potentially be early markers of CAD, and a few studies have tested this hypothesis (Table 3). In a nested case–control study in the prospective EPIC-Norfolk population study, baseline CXCL8 concentrations among 785 apparently healthy individuals, in whom fatal or nonfatal CAD developed during follow-up (average of 6 years), was significantly higher than in 1570 matched controls (3.5 pg/mL versus 3.1 pg/mL, \(P=0.001\)). The authors showed that people in the highest CXCL8 quartile had a fully adjusted odds ratio of 1.77 (95% CI, 1.21 to 2.60) compared with those in the lowest quartile (\(P=0.001\)). The odds ratio for future CAD was still significant after adjustment for traditional risk factors and after additional adjustment for CRP and total leukocyte count. Although the authors conclude that CXCL8 could represent a novel biomarker for CAD in apparently healthy individuals, there was a considerable overlap between the two study groups, and as discussed above, the levels were mostly just above the detection limit of the assay (ie, 2.5 pg/mL).

Deo et al observed strong associations with CAD risk factors such as older age, gender, hypertension, diabetes, and renal insufficiency after measuring CCL2 levels in subjects from the Dallas Heart Study (3499 subjects ≤65 years old).57 In this study, CCL2 levels were associated with coronary artery calcification (CAC), as a marker of subclinical atherosclerosis, in multivariable analyses adjusting for traditional coronary risk factors. However, when further adjustment was made for age, CCL2 was no longer independently associated with the presence of subclinical atherosclerosis. The authors conclude that CCL2 may not be useful as a clinical tool that is additive to the assessment of age, traditional risk factors, or CRP for the detection of subclinical atherosclerosis. More recently, Tang et al investigated the association between CCL2 levels and CAC in a large population-based sample

Table 1. Criteria for a Reliable Biomarker in Cardiovascular Disease

- Clear demonstration of incremental prognostic value over traditional risk models.
- Ability to reflect several pathogenic processes in atherogenesis and plaque destabilization.
- Stable levels in individuals. Not dependent on food intake with negligible circadian variation.
- High stability of the actual protein (e.g., long half-life).
- Easy to measure using inexpensive and standardized commercial assays with low variability.
- No requirement for specialized plasma/serum collection or storage.

Table 2. Challenges When Using Chemokines as Risk Predictors in Cardiovascular Disease

- Several chemokines are involved in atherogenesis, operating through different mechanisms and at different levels. No uniform chemokine marker that reflect all these processes.
- Several chemokines are found at low levels in serum/plasma; often below the detection limit of the actual assays.
- Short half-life. Unstable.
- Strict and bothersome demands for blood sampling protocol and storage (e.g., the tubes should be placed on ice, centrifuged within 30–60 minutes, stored at –70 to –80°C, and thawed less than three times).
- Release from platelets ex vivo during coagulation (serum) or during freeze and thaw cycles (ordinary plasma) may be an important confounder. Platelet-poor or platelet-free EDTA or citrated plasma should be preferred.
- Plasma/serum levels are influenced by the use of heparin in vivo.
free of clinical CAD (2246 whites and 470 blacks, mean age 55 years). Although CCL2 was significantly and positively associated with the presence of CAC after controlling for age and gender, with the same pattern in both white and blacks, the observed association was attenuated and no longer statistically significant after additionally adjusting for other CAD risk factors, further underscoring the limitation of CCL2 as an independent risk predictor in apparently healthy individuals. In line with this, Thakore et al could not detect any association between CCL2 levels and carotid intimal medial thickness (IMT) or stenosis in the Offspring Cohort of the Framingham Heart Study (n=2885, 53% women, mean age 52.3 years) incident CAD from the Framingham Heart Study (1984 to 2002). Finally, Herder et al investigated CCL2, CXCL8, and CXCL10 serum levels in a case–cohort design, based on data from 381 individuals with (294 men, 87 women, mean age 57.3 years) and 1977 individuals without (1006 men, 971 women, mean age 52.3 years) incident CAD from the prospective, population-based MONICA/KORA Augsburg study (1984 to 2002). The mean follow-up time was 11.0 years. Although baseline concentrations were significantly higher in cases compared with noncases (P<0.001 for all chemokines), only CCL2 and CXCL8 remained associated with CAD risk after adjustment for age and sex. Moreover, after adjustment for further cardiovascular and immunologic risk factors, the observed associations became nonsignificant. Taken together, although some significant findings, the ability of serum/plasma levels of chemokines to predict subclinical CAD is so far disappointing.

### Chemokines as Predictors of Cardiovascular Events in Patients With Overt CAD

In contrast to these somewhat disappointing studies on the ability of CCL2 to predict subclinical CAD, CCL2 may seem to be of some value in predicting cardiovascular event in patients with overt CAD (Table 3). In a substudy of the Oral Glycoprotein IIb/IIIa Inhibition with Orbofibin in Patients with Unstable Coronary Syndromes (OPUS-TIMI) 16 trial, the authors showed that among 2279 patients with ACS, CCL2 levels above the 75th percentile (238 pg/mL) were associated with an almost 2-fold increased risk of death or MI during follow-up (10 months) after adjustment for standard risk predictors including CRP. The authors suggest that CCL2 could be attractive as a surrogate biomarker in these patients and merits further study as a potential therapeutic target. More recently, the authors extended these findings by performing serial measurements of CCL2 in a large and well-characterized population of patients stabilized early after ACS (4244 individuals, 21 to 80 years, follow-up time 6 months to 2 years). The authors show that higher baseline levels of CCL2 were associated with an increased risk for long-term death and major adverse cardiac outcomes, independent of standard measurements such as creatinine clear-

### Table 3. Chemokine Plasma Levels and Risk for Cardiovascular Disease

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Population</th>
<th>Numbers</th>
<th>Follow Up</th>
<th>Assoc. disease/event</th>
<th>Study Design</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8/CXCL8</td>
<td>Healthy individuals</td>
<td>785 cases 1570 controls</td>
<td>6 years</td>
<td>Fatal and nonfatal CAD</td>
<td>Nested case-control</td>
<td>Higher levels of CXCL8 in cases</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>Healthy individuals</td>
<td>3499</td>
<td>Subclinical atherosclerosis</td>
<td>Cross sectional</td>
<td>CCL2 assoc. with CAD after adjusting for other risk factors</td>
<td></td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>Healthy individuals</td>
<td>2246 whites 470 Afr. Am.</td>
<td>Subclinical atherosclerosis</td>
<td>Cross sectional</td>
<td>No assoc. between CCL2 and CAD</td>
<td></td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>Healthy individuals</td>
<td>2885</td>
<td>Subclinical atherosclerosis</td>
<td>Cross sectional</td>
<td>No assoc. between CCL2 and carotid IMT</td>
<td></td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>CAD</td>
<td>381 cases 1977 controls</td>
<td>11 years</td>
<td>CAD</td>
<td>Case-cohort</td>
<td>No assoc. between CCL2 or CXCL8 and CAD after adjusting for other risk factors</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>ACS</td>
<td>2279</td>
<td>10 mo</td>
<td>Death or MI</td>
<td>Prospective</td>
<td>CCL2 above 75th percentile assoc. with increased risk of an event</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>ACS, stabilized</td>
<td>4244</td>
<td>6 mo – 2 years</td>
<td>Death, MI, stroke, HF</td>
<td>Prospective. Serial MCP-1 measures</td>
<td>CCL2 levels at baseline and at 4 mo assoc. with an event</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>ACS</td>
<td>183</td>
<td>13 mo</td>
<td>Composite (death, MI, unstable angina, revasc.)</td>
<td>Prospective</td>
<td>CCL2 predicted a new coronary event</td>
</tr>
<tr>
<td>Eotaxin-3/CCL2</td>
<td>CAD</td>
<td>1026</td>
<td>2.7-4.1 years</td>
<td>Cardiovascular death, MI</td>
<td>Prospective</td>
<td>Lower CCL26 levels predicted future events</td>
</tr>
<tr>
<td>RANTES/CCL5, IP-10/CXCL10, IL-8/CXCL8</td>
<td>CAD</td>
<td>312 cases 472 controls</td>
<td>Angiographically confirmed and stable CAD</td>
<td>Case control</td>
<td>Higher levels of CXCL8 and CXCL10, lower CCL5 in cases</td>
<td></td>
</tr>
<tr>
<td>RANTES/CCL5</td>
<td>Coronary angiography</td>
<td>389</td>
<td>24 mo</td>
<td>Cardiovascular death, MI</td>
<td>Prospective</td>
<td>Low CCL5 levels were an predictor of cardiac mortality</td>
</tr>
<tr>
<td>RANTES/CCL5</td>
<td>ACS</td>
<td>54</td>
<td>Refractory ischemia</td>
<td>Cross sectional</td>
<td>Higher CCL5 in ischemic versus stabilized patients</td>
<td></td>
</tr>
</tbody>
</table>

IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; IP-10, interferon (INF)–inducible protein of 10 kd; CAD, coronary artery disease; ACS, acute coronary syndrome; MI, myocardial infarction; IMT, intimal medial thickness; CAC, coronary artery calcium score; HF, heart failure.
ance and LDL cholesterol, as well as biomarkers such as CRP and B-type natriuretic peptide. In addition, CCL2 levels measured 4 months after ACS also provided independent prognostic value. Thus, while the authors confirmed that an CCL2 threshold of 238 pg/mL identifies patients at increased risk for adverse events in both the acute and the chronic setting after ACS, they also showed that patients with levels consistently above this threshold seemed to be at particularly high risk for death compared with those with persistently lower levels, or levels that were only transiently above this threshold. In contrast, and somewhat disappointingly, elevated CCL2 levels did not identify patients who derived incremental benefit from intensive statin therapy. The ability of CCL2 to predict coronary events in patients with overt CAD has also been reported by Kervinen et al showing in a much smaller study (n = 183) of unselected ACS patients, with a very high rate (64%) of coronary events (ie, cardiac death, recurrent MI, unstable angina, or revascularization) during follow-up (13 months), that increased plasma levels of CCL2, as well as the T cell marker soluble IL-2 receptor, were helpful for predicting new coronary events independent of other inflammatory mediators (ie, CRP and IL-6).63

Eotaxins (eotaxin-1/CCL11, eotaxin-2/CCL24, and eotaxin-3/CCL26) are members of the CC chemokine branch that mainly acts on CCR3-bearing cells like eosinophils, basophils, and lymphocytes of the T helper cell type 2 phenotype. There are few data on the role of these chemokines in CAD, but recently, Falcone et al reported that lower CCL26 concentrations were predictive of future cardiovascular events, whereas both CCL11 and CCL24 showed no association with risk, in a study population with confirmed CAD (n = 1,026, 841 with stable and 185 with unstable angina) and with 105 cardiac events during follow-up (2.7 to 4.1 years; Table 3).64 The highest risk of future cardiovascular events was observed in subjects with combined elevation of CCL5 during ex vivo coagulation attributable to markedly increased deposition of CCL5 on the vascular endothelium leading to greater CCR5 stimulation. However, the predictive value of low CCL5 levels may seem in contrast with a recent study by Kraaijeveld et al showing in a small population of patients with ACS (n = 54) that high plasma levels of CCL5 were significantly elevated in patients with refractory ischemic symptoms versus stabilized patients.70 There may be several reasons for these apparently discrepancies. First, platelets are the most important cellular source of circulating CCL5 levels, and the blood sampling protocol may have major influence on the estimated CCL5 level. Thus, although Kraaijeveld et al used platelet-free plasma, there is no detailed information on the blood sampling protocol in the other studies. Importantly, release from platelet ex vivo during freeze and thaw cycles may be decreased in those with active disease because of platelet degranulation in vivo, leading to underestimation of the actual CCL5 level. During meningococcal septicemia low serum levels of CCL5 are reported as a prominent feature,71 reflecting decreased release of CCL5 during ex vivo coagulation attributable to markedly enhanced release of CCL5 from activated platelets in vivo, illustrating that a decreased measurable CCL5 level under certain circumstances could reflect high rather than low CCL5 level in vivo. On the other hand, the use of heparin in ACS may lead to incidentally increased CCL5 levels attributable to enhanced release of heparan sulfate-bound chemokines in the vessel wall.55 However, the inability of CCL5 levels to predict long-term adverse cardiovascular outcomes in ACS may also reflect that the acute spike in CCL5 levels that occur in this particular setting is an unreliable marker of coronary inflammation and long-term prognosis. Nevertheless, these issues clearly illustrate the methodological challenges when using chemokines, and in particular those that are released from activated platelets, as risk predictors in CAD.

CCL5: High and Low Levels Are Associated With Disease Progression

Although most focus has been drawn against high levels of various chemokines, some studies have suggested that low levels of CCL5 may be associated with disease progression in atherosclerosis (Table 3). Thus, Rothenbacher et al reported that in 312 patients (aged 40 to 68 years) with angiographically confirmed and stable CAD and 472 age- and gender-matched controls, low levels of CCL5, as well as upregulation of CXCL10 and CXCL8, were associated with CAD, with no clear disease association for CCL2, macrophage inflammatory protein α (MIP-1α/CCL3), or CCL11.68 While data from such case-controlled study, with no longitudinal follow-up, should be interpreted with caution, Cavusoglu et al measured baseline plasma levels of CCL5 in 389 male patients undergoing coronary angiography at a Veterans Affairs Medical Center.69 The patients were followed-up prospectively for 24 months for the occurrence of cardiac mortality and MI. In the entire cohort of patients, low baseline CCL5 levels were an independent predictor of cardiac mortality. Furthermore, when patients were risk-stratified into those with and without an ACS, CCL5 was an independent predictor of both cardiac mortality and MI in those without an ACS. An additional group in whom low CCL5 levels were shown to be strongly predictive of events was the diabetic subset, possibly reflecting the greater atherosclerotic burden known to be present in this population. The authors hypothesize that the low CCL5 level in those with increased frequency of clinical events could reflect increased deposition of CCL5 on the vascular endothelium leading to greater CCR5 stimulation. However, the predictive value of low CCL5 levels may seem in contrast with a recent study by Kraaijeveld et al showing in a small population of patients with ACS (n = 54) that high plasma levels of CCL5 were significantly elevated in patients with refractory ischemic symptoms versus stabilized patients.70 There may be several reasons for these apparently discrepancies. First, platelets are the most important cellular source of circulating CCL5 levels, and the blood sampling protocol may have major influence on the estimated CCL5 level. Thus, although Kraaijeveld et al used platelet-free plasma, there is no detailed information on the blood sampling protocol in the other studies. Importantly, release from platelet ex vivo during freeze and thaw cycles may be decreased in those with active disease because of platelet degranulation in vivo, leading to underestimation of the actual CCL5 level. During meningococcal septicemia low serum levels of CCL5 are reported as a prominent feature,71 reflecting decreased release of CCL5 during ex vivo coagulation attributable to markedly enhanced release of CCL5 from activated platelets in vivo, illustrating that a decreased measurable CCL5 level under certain circumstances could reflect high rather than low CCL5 level in vivo. On the other hand, the use of heparin in ACS may lead to incidentally increased CCL5 levels attributable to enhanced release of heparan sulfate-bound chemokines in the vessel wall.55 However, the inability of CCL5 levels to predict long-term adverse cardiovascular outcomes in ACS may also reflect that the acute spike in CCL5 levels that occur in this particular setting is an unreliable marker of coronary inflammation and long-term prognosis. Nevertheless, these issues clearly illustrate the methodological challenges when using chemokines, and in particular those that are released from activated platelets, as risk predictors in CAD.

“New” Chemokines as Risk Predictor

Except for CCL5, the circulating levels of chemokines that have been studied as risk markers in CAD are rather low (ie, <500 pg/mL), and as for CXCL8, the levels are just above the detection limit of the assays, which weaken their impact as risk markers. Recently, Kraaijeveld et al showed that very high plasma levels (>100 ng/mL) of pulmonary and activation-regulated chemokine (PARC/CCL18) in ACS pa-
patients (n=54) were indicative of refractory symptoms, suggesting that this chemokine should be evaluated as a risk predictor in a larger population of CAD patients. Moreover, based on the link between inflammatory mediators and the cleavage of the membrane-bound to the soluble form of CXCL16, it has been suggested that soluble CXCL16 could serve as a reliable, stable, and robust marker of inflammation, being detectable in plasma/serum in concentrations regularly >1 ng/mL. Indeed, raised levels of soluble CXCL16 have been reported in some inflammatory conditions such as rheumatoid arthritis and systemic lupus erythematosus. However, conflicting data exist on plasma levels of CXCL16 in CAD. Thus, whereas Sheikine et al found decreased CXCL16 levels in both stable and unstable angina, others have reported increased CXCL16 levels in CAD patients, and in the study by Lehrke et al, particularly high levels were found in those with unstable disease. Hence, although some potential advantages (eg, relatively high serum/plasma levels, minor influence on freeze/thaw cycles, stable during storage; Aukrust P and Damås JK, unpublished data 2007), we are in need of bigger, carefully designed clinical studies evaluating CXCL16 as a potential biomarker of atherosclerotic vessel disease. At present, prospective studies on the association of CXCL16 with the risk of developing CAD or clinical events in those with overt CAD are lacking. Furthermore, recent studies have shown raised plasma levels of the so-called regulatory chemokines CCL19 and CCL21 in CAD with particularly high levels in those with unstable disease, and Erbel et al reported enhanced expression of CCL19 and CCL21 in unstable carotid and coronary plaques. However, at present, there are no prospective data on CCL19 and CCL21 as risks predictors in CAD. Finally, Ardigo et al have recently used a multimarker proteomic approach, hypothesizing that measuring serum levels of vascular derived inflammatory biomarkers could reveal a “signature of disease” that can serve as a highly accurate method to assess for the presence of CAD. By using this approach, they identified an optimal combined chemokine model as the best predictor of CAD (CCL11, CXCL10, CCL2, MCP-2/CCL8, MCP-3/CCL7, MCP-4/CCL13, and CCL3), far superior to CRP. Although few patients were examined in this cross-sectional study (49 cases and 44 controls), measuring of global patterns of cytokine and chemokine expression plausibly may yield more relevant biological information than individual protein assays. As several chemokines are involved in atherogenesis, at least partly through different mechanisms and at different levels, a combination of serum levels of multiple chemokines could potentially identify subjects with clinically significant atherosclerotic heart disease with a very high degree of accuracy. However, at present such an approach is unrealistic from a practical and clinical point of view.

Genetic Variation in the Chemokine Genes as Risk Factor in Atherogenesis

Epidemiological genetic studies in gene related to lipid metabolism have been of major importance for our understanding of the role of these factors in atherogenesis. Similar, polymorphism studies in chemokine or chemokine receptor genes, trying to relate these genetic variations to increased risk for cardiovascular disease, are of importance for the study of the pathogenic role of these mediators in the atherosclerotic process. Such analyses could also be of importance for risk stratification, in particular in the coming years where molecular methodology will be more accessible. Several chemokine/chemokine receptor polymorphisms have been linked to increased risk of cardiovascular disease such as polymorphisms in the gene for CCL2 and CCL5 and their corresponding receptors CCR2 and CCR5, respectively, as well as polymorphisms in the CX3CL1 receptor gene, CX3CR1. These issues have recently been reviewed and will not be discussed in further detail in this review. However, although the results from some of these studies may provide new conceptual, diagnostic, and therapeutic approaches to vascular diseases, the single nucleotide polymorphisms (SNPs) studies also have several limitations. Although the standards and quality seem to be improving, there is nevertheless a risk that SNP-based association analyses will squander academic trust and scientific resources owing to unsatisfactory analysis. Moreover, some of the chemokine/chemokine receptor genotypes are rare, making epidemiological conclusions difficult except in the largest cohorts. However, the strength of such studies will greatly improve if the actual polymorphism could be related to phenotypic alterations with relevance to atherogenesis. As an elegant and path-breaking example, McDermott et al showed that CX3CL1-dependent cell–cell adhesion under conditions of physiological shear was severely reduced in cells expressing the CX3CR1 polymorphism which causes amino acid change from threonine to methionine at position 280 (CX3CR1-M280). This was associated with marked reduction in the kinetics of CX3CL1 binding as well as reduced CX3CL1-induced chemotaxis of primary leukocytes from donors homozygous for CX3CR1-M280. Importantly, these authors also showed that CX3CR1-M280 is independently associated with a lower risk of atherosclerotic cardiovascular disease in the Offspring Cohort of the Framingham Heart Study, a long-term prospective study of the risks and natural history of this disease (204 cases and 1655 controls).

Chemokines as Predictor Restenosis and Cardiac Allograft Vasculopathy

In addition to atherosclerosis, chemokines are also involved in the pathogenesis of other and related cardiovascular disorders such as the arterial remodeling process characterizing restenosis after PCI, with and without stenting, and cardiac allograft vasculopathy (CAV) after heart transplantation. The arterial vessel wall response to a variety of injuries consists in structural changes, which can result in luminal narrowing and aggravation of the underlying disease. This arterial remodeling is characterized by neointima formation and medial thickening, inflammatory cell recruitment, and endothelial dysfunction. Chemokines and the corresponding receptors have been shown to participate at every step of the remodeling process. Thus, CCL2 may promote monocyte infiltration of the injured vessel wall and can stimulate proliferation of SMC in models of restenosis, and indeed, there are some reports suggesting that CCL2 can predict restenosis after PCI, with and without accompanying stent-
Table 4. Types of Studies Needed to Move Chemokine Measurements into the Clinic

- Studies in patients with established CAD or patients with acute coronary syndromes should be given priority.
- Prospective studies including a large number of patients, focusing on clinical end-point.
- Studies that examine the relationship between chemokine levels and the degree of atherosclerosis and plaque instability as assessed by new imaging techniques, including molecular imaging, will also be needed.
- Studies that investigate the ability of combined measurements of several chemokines to give prognostic information (signature of disease).
- Studies that include serial measurements in addition to baseline measurements.
- Studies that examine the predictive value of “new” chemokines with high serum/plasma concentrations and with presumably more stability (e.g. CXCL16, CCL18, and CCL21).
- Studies that examine the ability of chemokines to monitor responses to therapeutic intervention trials.
- Studies should compare the predictive value of chemokines with the predictive value of established biomarkers including CRP.

Conclusions

The role of chemokines as clinical biomarkers is at present unclear, and their ability to predict subclinical atherosclerosis has been disappointing. Further prospective studies, including a larger number of patients, are needed to make any firm conclusion (Table 4). Such studies should also include serial measurements in addition to baseline measurements, and based on previous research, studies in patients with established CAD or ACS should be given priority as opposed to studies in apparently healthy individuals. Studies that examine the relationship between chemokine levels and the degree of atherosclerosis and plaque instability as assessed by new imaging techniques, including molecular imaging, will also be needed. While the demonstration of an association between chemokines and CAD is a necessary first step, such studies do not establish the full clinical utility of a biomarker, which is a more demanding process that requires validation in multiple cohorts, a reliable and cost-effective assay, and clear demonstration of incremental prognostic value over traditional risk models. If successful, such new biomarkers will be useful indicators for better risk assessment, diagnosis, and prognosis, as well as for monitoring pharmacological treatments for atherosclerosis. Based on the participation of several chemokines in atherogenesis, it is possible that in the future, combined measurements of multiple chemokines could reveal as a “signature of disease” that can serve as a highly accurate method to assess for the presence of atherosclerotic disease. Finally, it is also important to underscore that the lack of function as a biomarker does not exclude an important pathogenic role of chemokines in atherogenesis and plaque destabilization, and accordingly, does not negate the potential value of chemokines as novel targets for therapy in atherosclerotic disorders.

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