

## Theme Issue Article

# Chemokines in cardiovascular risk prediction

Pål Aukrust<sup>1,2</sup>, Arne Yndestad<sup>1</sup>, Camilla Smith<sup>1</sup>, Thor Ueland<sup>1,3</sup>, Lars Gullestad<sup>4</sup>, Jan K. Damås<sup>1,2</sup>

<sup>1</sup>Research Institute for Internal Medicine, <sup>2</sup>Section of Clinical Immunology and Infectious Diseases, <sup>3</sup>Section of Endocrinology, and <sup>4</sup>Department of Cardiology, Rikshospitalet-Radiumhospitalet, Medical Center, University of Oslo, Oslo, Norway

### Summary

In consideration of the important role of inflammation in plaque progression and stability, recent work has focused on whether plasma markers of inflammation can non-invasively diagnose and predict coronary artery disease (CAD) and other forms of atherosclerotic disorders. Although several studies support an important pathogenic role of chemokines in atherogenesis and plaque destabilization, 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 up-stream inflammatory activity, stable levels in individuals and high stability of the actual protein (e.g. 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 (e.g. interleukin 8 and monocyte chemoattractant protein-1) have been shown to be predictive for future cardiac

events in some studies, independent of traditional cardiovascular risk factors and C-reactive protein, and although certain gene polymorphisms of chemokines/chemokine receptors (e.g. fractalkine receptor) have been shown to be predictive of future atherosclerotic disease, further prospective studies, including a larger number of patients, are needed to make any firm conclusion. While the demonstrations of an association between chemokines and CAD are 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, and clear demonstration of incremental prognostic value over traditional risk models. If successful, such new biomarker will be a useful indicator for better risk assessment, diagnosis, and prognosis, as well as monitoring pharmacological treatments for atherosclerosis.

### Keywords

Chemokines, atherosclerosis, gene polymorphism, biomarkers, inflammation

**Thromb Haemost 2007; 97: 748–754**

## Introduction

Cardiovascular disease remains a leading cause of death throughout the world despite advances in its detection and treatment. Commonly used risk algorithms, such as the Framingham Risk Score and lipid parameters fail to identify all affected individuals. 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 (e.g. statins) in these patients.

## Inflammatory mediators as biomarkers in cardiovascular disease

In consideration of the important role of inflammation in plaque progression and stability, recent work has focused on whether plasma markers of inflammation can non-invasively diagnose and predict coronary artery disease (CAD) and other forms of atherosclerotic disorders (1). Numerous studies have shown that inflammatory markers can help in identifying patients with stable CAD and acute coronary syndromes (ACS), as well as being predictive for development of CAD in high-risk patients (2). Moreover, several inflammatory markers improve risk stratification in CAD patients, being independent prognostic markers for cardiovascular events (2, 3). C-reactive protein (CRP), the prototype inflammatory marker, has certainly been the best

Correspondence to:

Pål Aukrust  
Section of Clinical Immunology and Infectious Diseases, Medical Department  
Rikshospitalet-Radiumhospitalet Medical Center, University of Oslo  
Sognsvannsveien 20, 0027 Oslo, Norway  
Tel.: +47 23073628, Fax: +47 23073630  
E-mail: pal.aukrust@rikshospitalet.no

Financial support:

This work was supported by grants from the Norwegian Council of Cardiovascular Research, Research Council of Norway, the University of Oslo, Medinnova Foundation, Rikshospitalet-Radiumhospitalet Medical Center, and Helse Sør.

Received January 16, 2007

Accepted after revision February 15, 2007

Prepublished online April 4, 2007

doi:10.1160/TH07-01-0029

studied of these „new“ biomarkers. CRP has proven remarkably robust as a marker of cardiovascular risk and gives predictive value beyond that of traditional risk factors in CAD patients (3, 4). Several large-scale studies have shown baseline levels of CRP to independently predict future myocardial infarction (MI), stroke, and cardiovascular death in apparently healthy individuals (3, 4). Moreover, among patients with stable angina and established CAD, plasma levels of CRP have consistently been shown associated with risk of cardiovascular events (3, 4). Also, in patients with ACS, CRP levels seem to give prognostic information on mortality, beyond that of troponin levels, but seem to have no relation with the early or late occurrence of MI (5). Finally, measuring CRP in CAD patients may be useful in monitoring responses to various interventions such as statin therapy (6). 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 (7). Indeed 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 (e.g. interleukin [IL]-6, intracellular adhesion molecule [ICAM]-1, osteoprotegerin, and soluble CD40 ligand [sCD40L]) (8–11).

## Chemokines – major pathogenic role in atherogenesis and plaque destabilization

Chemokines are a family of chemotactic cytokines characterized by their ability to cause directed migration of leukocytes into inflamed tissue, and raised levels are found in several inflammatory disorders including CAD (12). Moreover, these chemotactic cytokines seem not only to be raised in circulation, but also within the atherosclerotic lesions (13). Hence, there are several reports of enhanced expression of CXC-chemokines (e.g. IL-8, neutrophil-activating peptide [NAP]-2, CXCL16, and interferon- $\gamma$ -inducible 10 [IP-10]), CC-chemokines (e.g. monocyte chemoattractant protein [MCP]-1, leukotactin-1 [Lkn-1], and regulated upon activation, normal T-cell expressed and secreted [RANTES]) as well as some of their corresponding chemokine receptors within human atherosclerotic lesions (12–15). In addition to being potent chemoattractants, several other leukocyte responses such as cell proliferation, enzyme secretion and induction of reactive oxygen species, have been observed *in vitro* after chemokine stimulation (13). Moreover, beyond their effects on leukocytes, chemokines may also interfere with smooth muscle cell (SMC) migration and growth, as well as platelet activation (16, 17). Some of these responses may clearly be relevant to atherogenesis and plaque destabilization, and indeed, the co-expression of chemokines and their receptor within atherosclerotic lesions, involving various cell types such as T cells, macrophages, and vascular SMC, suggests their involvement not only in the regulation of lymphocyte recruitment into atherosclerotic lesions, but also in other processes with relevance to atherogenesis such as regulation of vascular SMC phenotype (13). Moreover, recent studies *in vivo* have shown that targeted disruption of the genes for MCP-1, CCR2 (i.e. MCP-1 receptor) and CXCR2

(i.e. IL-8 receptor) significantly decreases atherosclerotic lesion formation and lipid deposition in mice prone to develop atherosclerotic lesions (18–20). Also, two independent reports recently indicated the involvement of the CXC chemokine fractalkine (also known as CX3CL1) in atherogenesis, showing that CX3CR1 deficiency (i.e. the fractalkine receptor) decreased atherosclerosis in animal models (21, 22). These and other studies in gene-modified mice strongly suggest an important pathogenic role of chemokines in atherogenesis.

Notably, infiltration and activation of circulating T cells and monocytes into the atherosclerotic plaque may also be involved in the triggering of ACS (23). Again, chemokines may play an important role in this immune-mediated plaque destabilization, not only by recruiting activated leukocytes into the atherosclerotic vessel wall, but also by directly contributing to plaque rupture and thrombus formation by enhancing the matrix degrading potential in macrophages, by inducing tissue factor (TF) and matrix metalloproteinases (MMPs) in vascular SMC, and by promoting neovascularization within the atherosclerotic lesion which in turn may act as a conduit for the entry of leukocytes into sites of chronic inflammation (24–29). Chemokines could also promote plaque rupture by enhancing oxidative stress and apoptosis within the atherosclerotic lesions. In fact, angina patients have been found to have raised levels of both CC- and CXC-chemokines with particularly high concentration of IL-8, MCP-1, and macrophage inflammatory protein (MIP)-1 $\alpha$  in unstable disease, significantly correlated with enhanced oxidative stress in these patients (30). Consequently, chemokine receptors/ligands could be identified as potential important pathogenic mediators not only in the chronic atherosclerotic process, but also in plaque destabilization with subsequent development of ACS. Such a notion was recently supported in a study by Lutgens et al. showing that an inhibiting antibody for MCP-1 and MCP-5 induced a stable plaque phenotype in ApoE<sup>-/-</sup> (31).

## 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 these chemotactic cytokines 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. Moreover, CRP has a long half-life, exhibits stable levels in individuals, and has negligible circadian variation (32, 33). It is easily measured, and inexpensive standardized assays provide similar results in fresh, stored, or frozen plasma, reflecting the high stability of the protein (32, 33). All these characteristics are important criteria for an ideal biomarker in cardiovascular disease. Unfortunately, measurements of chemokines in plasma/serum are hampered by some

methodological problems which may limit their use as biomarkers in CAD and other cardiovascular disease. First, platelets are an important cellular source for several chemokines such as RANTES, NAP-2, and epithelial neutrophil-activating peptide (ENA)-78 (34), and these parameters should therefore ideally be measured in platelet-poor or platelet-free plasma which is not always available. Thus, measurement in serum may underestimate the concentration in high-risk patients (i.e. less release of chemokines from platelets during coagulation *ex vivo* due to degranulation and platelet activation *in vivo*). Moreover, freeze and thaw cycles of ordinary plasma samples may result in *ex-vivo* release of chemokines from platelets which again may depend on platelet counts as well as the degree of platelet degranulation *in vivo*. Second, plasma/serum levels of several chemokines are in general low, often just above the detection limit of the various enzyme immunoassays, and these aspects may limit their use as a reliable biomarkers. For example, while serum levels of IL-8 in CAD patients are mostly <10 pg/ml, CRP levels in these individuals will mostly be >1 µg/ml (5, 6, 30, 35). Finally, heparin administration may influence plasma/serum levels of chemokines through enhanced release of heparan sulphate-bound chemokines in the vessel wall, which may limit their use as prognostic markers in ACS (35).

## Chemokines as markers for subclinical CAD

Migration of monocytes into the arterial wall is an early event in atheroma formation (1). Based on the suggested important role of chemokines in this process, and in particular IL-8 and MCP-1 (18–20), they could potentially be early markers of CAD, and a few studies have tested this hypothesis (Table 1). In a nested case-

control study in the prospective EPIC-Norfolk population study, baseline IL-8 concentrations among 785 apparently healthy individuals, in whom fatal or non-fatal CAD developed during follow-up (average of ~6 years), was significantly higher than in 1,570 matched controls (3.5 pg/ml vs. 3.1 pg/ml,  $p=0.001$ ) (36). The odds ratio (OR) for future CAD was still significant after adjustment for traditional risk factors and after additional adjustment for CRP and white cell count. Although the authors conclude that IL-8 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 (i.e. 2.5 pg/ml).

Deo et al. observed strong associations with CAD risk factors such as older age, female sex, hypertension, diabetes, and renal insufficiency after measuring MCP-1 levels in subjects from the Dallas Heart Study (3,499 subjects <65 years old) (37). In this study, MCP-1 was associated with coronary artery calcium score in multivariable analyses adjusting for traditional coronary risk factors. However, when further adjustment was made for age, MCP-1 was no longer independently associated with the presence of subclinical atherosclerosis. The authors conclude that these results suggest that MCP-1 may not be useful as a clinical tool that is additive to the assessment of age, traditional risk factors, and/or CRP for the detection of subclinical atherosclerosis.

## Chemokines as markers of established cardiovascular disease

A few case-control studies have examined the ability of chemokines to predict established atherosclerotic disease (Table 1). In

**Table 1: Studies [references] on plasma/serum levels of chemokines as risk predictors for cardiovascular disease.**

Chemokine	Population	Numbers	Follow-up	Associated disease/event	Study design	Outcome
IL-8 (36)	Healthy individuals	785 cases 1,570 controls	6 years	Fatal and nonfatal CAD	Nested case-control	Higher levels of IL-8 in cases
MCP-1 (37)	Healthy individuals	3,499		Subclinical atherosclerosis	Cross sectional	MCP-1 associated with coronary artery calcium score
MCP-1 (38)	CAD/PAD	412/209 cases 733/709 controls		CAD/PAD	Case control	Higher levels of MCP-1 in cases
IL-8, IP-10, MCP-1, RANTES, MIP-1 $\alpha$ , eotaxin (39)	CAD	312 cases 472 controls		Angiographically confirmed and stable CAD	Case control	Higher levels of IL-8 and IP-10, lower RANTES in cases
MCP-1 (40)	ACS	2,270	10 months	Death or MI	Prospective	MCP-1 levels above 75 <sup>th</sup> percentile associated with increased event risk
MCP-1 (41)	ACS	183	13 months	Composite (death, MI, unstable angina, revascularization)	Prospective	MCP-1 predicted a new coronary event
Eotaxin-3 (42)	CAD	1,026	2.7–4.1 years	Cardiovascular death, MI	Prospective	Lower eotaxin-3 levels predicted future events

IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; IP-10, interferon (INF)-inducible protein of 10 kd; MIP-1 $\alpha$ , macrophage inflammation protein-1; CAD, coronary artery disease; PAD, peripheral artery disease; ACS, acute coronary syndrome; MI, myocardial infarction.

the Atherosclerosis Risk in Communities (ARIC) study, 209 cases with lower extremity peripheral artery disease (PAD) and 412 cases with incident CAD were compared with 733 and 709 subjects without PAD and CAD, respectively (38). Participants with incident CAD and in particular those with PAD had significantly higher levels of MCP-1 than controls. MCP-1 levels in the upper versus lower tertile were associated with PAD, independent of traditional CAD risk factors. However, this association seemed not to be independent of CRP, which weakens the usefulness of MCP-1 as a reliable inflammatory biomarker in this population. Moreover, in another case-control study, Rothenbacher et al. included 312 patients, aged 40 to 68 years, with angiographically confirmed and stable CAD and 472 age- and gender-matched controls (39). The authors found no universal upregulation of chemokines in CAD patients as compared to controls, with up-regulation of IP-10 and IL-8 versus down-regulation of RANTES, and no clear disease association for MCP-1, MIP-1 $\alpha$ , or eotaxin. Moreover, the association with CAD was attenuated or lost when carrying out multivariate adjustments for a variety of covariates, even if CRP was not included in these models.

## Chemokines as predictors of cardiovascular events in patients with overt CAD

In contrast to these somewhat disappointing case-control studies, MCP-1 seems to be of some value in predicting cardiovascular event in patients with ACS (Table 1). In a substudy of the Oral Glycoprotein IIb/IIIa Inhibition with Orbofiban in Patients with Unstable Coronary Syndromes (OPUS-TIMI) 16 trial, the authors showed that among 2,279 patients with ACS, MCP-1 levels above the 75<sup>th</sup> percentile (238 pg/ml) were associated with an almost two-fold increased risk of death or MI during follow-up (10 months) after adjustment for standard risk predictors including CRP (40). The authors suggest that MCP-1 could be attractive as a surrogate biomarker in these patients and merits further study as a potential therapeutic target. A similar finding 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 (i.e. cardiac death, recurrent MI, unstable angina, or revascularization) during follow-up (13 months), that increased plasma levels of MCP-1, as well as the T-cell marker soluble IL-2 receptor, were helpful for predicting new coronary events independent on other inflammatory mediators (i.e. CRP and IL-6) (41).

Eotaxins (eotaxin, eotaxin-2, and eotaxin-3) are members of the CC chemokine branch that mainly act on CCR3-bearing cells like eosinophils, basophils, and lymphocytes of the T-helper cell type 2 (Th2) phenotype (12). There are few data on the role of these chemokines in CAD, but recently Falcone et al. reported that lower eotaxin-3 concentrations were predictive of future cardiovascular events, whereas both eotaxin and eotaxin-2 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 (2.7–4.1 years) (42) (Table 1). The highest risk of future cardiovascular events was observed in subjects with combined elevation of CRP and reduction of eotaxin-3, and receiver-operating-characteristic curves analysis suggested a superior prognostic value of eotaxin-3 compared with CRP for predicting cardiac events. However, although there are some reports of potential anti-inflammatory effects of eotaxin-3 (42), the reason for the association between low eotaxin-3 levels and cardiac events remain obscure. The authors have previously reported an association between high eotaxin levels and documented CAD, further underscoring such a notion (43).

## Genetic variation in the chemokine genes as a risk factor for atherosclerosis

Epidemiologic genetic studies in genes related to lipid metabolism have been of major importance for our understanding of the role of these factors in atherogenesis. Similarly, polymorphism studies in chemokine or chemokine receptor genes, trying to relate these genetic variations to increased risk for cardiovascular disease (Table 2), are of importance for the study of the pathogenic role of these mediators in the atherosclerotic process (44). Moreover, such analyses could also be of importance for

**Table 2: Studies [references] on polymorphisms in chemokine and chemokine receptor genes and risk for cardiovascular disease.**

Gene	Mutations	Populations	Associated disease/clinical events
MCP-1 (45–49)	–2518G, G-927C, A-2578G	Healthy individuals, CAD, stroke HIV patients	–2518G, G-927C and A-2578G variants in homozygous form appears as a genetic risk factor for IMT, CAD, occult ischemia and MI
CCR2 (50,51)	V64I	CAD/MI	V64I is associated with a higher prevalence of MI. However, data is conflicting regarding this rare allele.
CCR5 (51,53)	CCR5 $\Delta$ 32	CAD/MI	CCR5 $\Delta$ 32 allele seems to protect against an early episode of MI
RANTES (45,54,55)	–28G, –403A	CAD, DM type II	–403A allele is associated with CAD, cardiac events and all cause mortality
CX3CR1 (57–59) CX3CR1 (60)	CX3CR1-I249, CX3CR1-M280	CAD, ACS	CX3CR1–I249 and CX3CR1-M280 alleles are associated with a markedly reduced risk of CAD and ACS and improved endothelium-dependent vasodilation. One study (60) suggests protective effect of the M280 and harmful effect of the I249 polymorphism on the occurrence of ACS.

MCP-1, monocyte chemoattractant protein-1; CCR2, –5, CC-chemokine receptor 2, –5; CX3CR1, CX3C receptor 1; CAD, coronary artery disease; HIV, human immunodeficiency virus; MI, myocardial infarction; DM, diabetes mellitus; ACS, acute coronary syndrome; IMT, intima-media thickness.

risk stratification, in particular in the coming years where such molecular methodology will be more accessible.

## Polymorphisms in the genes for MCP-1 and its receptor CCR2

A polymorphism in the promoter of MCP-1 (the substitution of G for A at position -2518) has been associated with increased transcription of the *MCP-1* gene, and Szalai et al. found that the frequency of the -2518G homozygote variant was significantly higher in 318 CAD patients referred to coronary bypass surgery, than in 320 controls, significantly associated with elevated lipoprotein (a) levels, a known risk factor for CAD (45). This MCP-1 polymorphism was also shown to independently predict occult ischemia in an apparently healthy high risk population (679 apparently healthy 24- to 59-year-old siblings of people with premature CAD) (46). Interestingly, this -2518G polymorphism has also been found to be associated with a five-fold increased risk for atherosclerosis in HIV-infected individuals, as assessed by ultrasonography (47). Moreover, in a recent study of 470 patients with ischemic stroke, Brenner et al. found an association between the occurrence of two other MCP-1 polymorphisms (i.e. MCP-1 G-927C and MCP-1 A-2578G) and the common carotid artery intima media thickness (48). One of these polymorphisms (i.e. A-2578G), was recently found to be associated with higher serum levels of MCP-1 and higher prevalence of MI in the Framingham heart study, also after adjustment for other CAD risk factors (49). This latter study, trying to relate a gene polymorphism not only to the prevalence of cardiac events, but also to phenotypic MCP-1 alterations (i.e. raised serum levels) in a relatively large study population (n=1,609), may be of particularly importance. In contrast to these associations between MCP-1 polymorphism and atherosclerotic disorders, the relation between the polymorphism of the MCP-1 receptor *CCR2* gene, in which the valine at amino acid 64 in the first transmembrane domain is replaced with isoleucine (V64I), and CAD is unclear (44, 50, 51). This may at least partly reflect that the mutant *CCR2*-V64I protein has not been shown to be functionally defective or to have different levels of expression (44), underscoring the importance of relating gene polymorphisms to functional alterations.

## Polymorphism in the genes for RANTES and its receptor CCR5

A 32-base-pair deletion in the *CCR5* receptor (*CCR5*Δ32) and two promoter polymorphisms in *RANTES* (-28 C to G and -403 G to A) have been identified. The 32-base-pair deletion in *CCR5* encodes a protein that is severely truncated and cannot be detected at the cell surface (52). Taking patients with their first MI at the age of >60 years as the reference group (n=96), Gonzalez et al. reported that non-carriers of the Δ32*CCR5*-allele, in a study population of 214 patients with an age at the first MI episode <55 years, would have a three-fold higher risk of suffering from such an episode at this age (51). Szalai et al. also suggested that the *CCR5* Δ32-genotype was protective against CAD because they found no *CCR5*Δ32 homozygotes in CAD patients

(n=318) (45). However, the frequency of this *CCR5* polymorphism was rather low even in the control group (6 out of 320), suggesting that the Δ32 deletion could not be used for risk stratification. Moreover, in the Nurses' Health Study, studying 248 female cases (non fatal MI and fatal CAD) and 496 controls, the authors found no association between the *CCR5*Δ32 polymorphism or five other *CCR5* polymorphisms and the risk for CAD (53). However, as in the study of Gonzalez et al. (51), they found a strong inverse association for certain *CCR5* variants (i.e. Δ32 deletion) and early age of CAD onset.

*RANTES* is one of the *CCR5* ligands that have been linked to atherogenesis. While Szalai et al. found no association between the *RANTES* polymorphisms -28G and -403A and CAD, at least partly reflecting the relatively low numbers of individuals with these polymorphisms (45), Simeoni et al. reported that the 403A polymorphism was associated with coronary atherosclerosis, independently from conventional risk factors and CRP or fibrinogen as inflammatory biomarkers (54). Specifically, the A allele frequency was higher in 2,694 cases with coronary atherosclerosis compared to 530 controls free of this vascular disorder. Moreover, Boger et al. reported that the 403A polymorphism was associated with all-cause mortality, mainly due to cardiac events, in patients with type 2 diabetes mellitus and end stage renal disease (55).

## Polymorphisms in the fractalkine receptor (CX3CR1) gene

Two polymorphisms have been identified in *CX3CR1*; one which causes a codon change from valine to isoleucine at position 249 (*CX3CR1*-I249) and another that causes a codon change from threonine to methionine at position 280 (*CX3CR1*-M280) (56). These changes are located in the sixth and seventh transmembrane domains, respectively. At least four important studies on the association between *CX3CR1* polymorphisms and atherosclerosis have been published. First, when *CX3CR1* genotypes were analyzed in 151 patients with ACS and in 249 healthy controls, Moatti et al. found that *CX3CR1* I249 heterozygosity was associated with a markedly reduced risk of acute coronary events, independent of established acquired coronary risk factors (57). In another study, genotyping of the *CX3CR1*-V249I polymorphism was performed in a cohort of 339 white individuals who underwent cardiac catheterization (197 with and 142 without CAD), showing that the *CX3CR1* I249 allele was associated with decreased risk of CAD and improved endothelium-dependent vasodilatation (58). Third, McDermott et al. showed that *CX3CL1*-dependent cell-cell adhesion under conditions of physiologic shear was severely reduced in cells expressing *CX3CR1*-M280 (59). 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 1,655 controls) (59).

This latter study, reporting association between *CX3CR1* coding polymorphisms and various atherosclerosis end points in the Framingham Offspring Cohort contrasts with most association studies principally because the authors included functional evaluation of the actual polymorphisms. Finally, a recent cross-sectional study by Niessener et al., analyzing 720 patients with verified CAD, suggests a more complex interaction between the two *CX3CR1* polymorphisms showing protective effect of the M280 polymorphism and a harmful influence of the I249 polymorphism on the occurrence of ACS (60).

## Limitations of the polymorphism studies

Will the presence of the homozygous state for certain chemokine/chemokine receptor polymorphism come to be viewed as a standard cardiovascular risk factor? The tools and general knowledge that are necessary to approach these issues appear to be available. The results from some of these studies may provide new conceptual, diagnostic, and therapeutic approaches to vascular diseases. However, the single nucleotide polymorphisms (SNPs) studies also have several limitations (61). 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. Furthermore, the strength of such studies will greatly improve if the actual polymorphism could be related to phenotypical alterations with relevance to atherogenesis.

## Conclusions

Although several studies support an important pathogenic role of chemokines in atherogenesis and plaque destabilization, potentially representing attractive therapeutic targets in atherosclerotic heart disease, their role as clinical biomarkers is unclear, and further prospective studies, including a larger number of patients, are needed to make any firm conclusion. 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, however, such new a biomarker will be a useful indicator for better risk assessment, diagnosis, and prognosis, as well as for monitoring pharmacological treatments for atherosclerosis.

## References

- Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352: 1685–1695.
- Fichtlscherer S, Heeschen C, Zeiher AM. Inflammatory markers and coronary artery disease. *Curr Opin Pharmacol* 2004; 4: 124–131.
- Tsimikas S, Wilerson JT, Ridker PM. C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. *J Am Coll Cardiol* 2006; 47 (8 Suppl): C19–31.
- Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 2003; 107: 363–369.
- Morrow DA, Rifai N, Antman EM, et al. C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI 11A substudy. *Thrombolysis in Myocardial Infarction. J Am Coll Cardiol* 1998; 31: 1460–1465.
- Morrow DA, de Lemos JA, Sabatine MS, et al. Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor Trial. *Circulation* 2006; 114: 281–288.
- Ridker PM, Hennekens CH, Buring JE, et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000; 342: 836–843.
- Biasucci LM, Liuzzo G, Fantuzzi G, et al. Increasing levels of interleukin (IL)-1Ra and IL-6 during the first 2 days of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. *Circulation* 1999; 99: 2079–2084.
- Ridker PM, Hennekens CH, Roitman-Johnson B, et al. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *Lancet* 1998; 351: 88–92.
- Ueland T, Jemmland R, Godang K, et al. Prognostic value of osteoprotegerin in heart failure after acute myocardial infarction. *J Am Coll Cardiol* 2004; 44: 1970–1976.
- Heeschen C, Dimmeler S, Hamm CW, et al.; CAPTURE Study Investigators. Soluble CD40 ligand in acute coronary syndromes. *N Engl J Med* 2003; 348: 1104–1111.
- Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 2006; 354: 610–621.
- Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* 2006; 86: 515–581.
- Smith C, Damàs JK, Otterdal K, et al. Increased levels of Neutrophil-activating peptide-2 in acute coronary syndromes. Possible role of platelet-mediated vascular inflammation. *J Am Coll Cardiol* 2006; 48: 1591–1599.
- Lee WH, Kim SH, Jeong EM, et al. A novel chemokine, Leukotactin-1, induces chemotaxis, pro-atherogenic cytokines, and tissue factor expression in atherosclerosis. *Atherosclerosis* 2002; 161: 255–260.
- Schechter AD, Berman AB, Taubman MB. Chemokine receptors in vascular smooth muscle. *Microcirculation* 2003; 10: 265–272.
- Weber C. Platelets and chemokines in atherosclerosis: partners in crime. *Circ Res* 2005; 96: 612–616.
- Gu L, Okada Y, Clinton SK, et al. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol Cell* 1998; 2: 275–281.
- Boring L, Gosling J, Clearl M, et al. Decreased lesion formation in *CCR2*<sup>-/-</sup> mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature* 1998; 394: 894–897.
- Boisvert WA, Santiago R, Curtiss LK, et al. A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest* 1998; 101: 353–361.
- Combadiere C, Potteaux S, Gao JL, et al. Decreased atherosclerotic lesion formation in *CX3CR1*/apolipoprotein E double knockout mice. *Circulation* 2003; 107: 1009–1016.
- Lesnik P, Haskell CA, Charo IF. Deceased atherosclerosis in *CX3CR1*<sup>-/-</sup> mice reveals a role for fractalkine in atherogenesis. *J Clin Invest* 2003; 111: 333–340.
- Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 2001; 104: 365–372.
- Moreau M, Brocheriou I, Petit L, et al. Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: relevance to stability of atherosclerotic plaque. *Circulation* 1999; 99: 420–426.
- Schechter AD, Rollins BJ, Zhang YJ, et al. Tissue factor is induced by monocyte chemoattractant protein-1 in human aortic smooth muscle and THP-1 cells. *J Biol Chem* 1997; 272: 28568–28573.
- Haque, Fallon JT, Pan JJ, et al. Chemokine receptor-8 (CCR8) mediates human vascular smooth muscle cell chemotaxis and metalloproteinase-2 secretion. *Blood* 2004; 103: 1296–1304.
- Kodali R, Hajjou M, Berman A, et al. Chemokines induce matrix metalloproteinase-2 through activation of epidermal growth factor receptor in arterial smooth muscle cells. *Cardiovasc Res* 2006; 69: 706–715.
- Moulton KS, Vakili K, Zurakowski D, et al. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. *Proc Natl Acad Sci USA* 2003; 100: 4736–4741.
- Charo IF, Taubman MB. Chemokines in the pathogenesis of vascular disease. *Circ Res* 2004; 95: 858–866.
- Aukrust P, Berge RK, Ueland T, et al. Interaction between chemokines and oxidative stress: possible pathogenic role in acute coronary syndromes. *J Am Coll*

- Cardiol 2001; 37: 485–491.
31. Lutgens E, Faber B, Schapira K, et al. Gene profiling in atherosclerosis reveals a key role for small inducible cytokines: validation using a novel monocyte chemoattractant protein monoclonal antibody. *Circulation* 2005; 111: 3443–3452.
  32. Ockene IS, Matthews CE, Rifai N, et al. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. *Clin Chem* 2001; 47: 444–450.
  33. Meier-Ewert HK, Ridker PM, Rifai N, et al. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. *Clin Chem* 2001; 47: 426–430.
  34. Weyrich AS, Zimmerman GA. Platelets: signaling cells in the immune continuum. *Trends Immunol* 2004; 25: 489–495.
  35. Ranjbaran H, Wang Y, Manes TD, et al. Heparin displaces interferon-gamma-inducible chemokines (IP-10, I-TAC, and Mig) sequestered in the vasculature and inhibits the transendothelial migration and arterial recruitment of T cells. *Circulation* 2006; 114: 1293–1300.
  36. Boekholdt SM, Peters RJ, Hack CE, et al. IL-8 plasma concentrations and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. *Arterioscler Thromb Vasc Biol* 2004; 24: 1503–1508.
  37. Deo R, Khera A, McGuire DK, et al. Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. *J Am Coll Cardiol* 2004; 44: 1812–1818.
  38. Hoogeveen RC, Morrison A, Boerwinkle E, et al. Plasma MCP-1 level and risk for peripheral arterial disease and incident coronary heart disease: Atherosclerosis Risk in Communities study. *Atherosclerosis* 2005; 183: 301–307.
  39. Rothenbacher D, Muller-Scholze S, Herder C, et al. Differential expression of chemokines, risk of stable coronary heart disease, and correlation with established cardiovascular risk markers. *Arterioscler Thromb Vasc Biol* 2006; 26: 194–199.
  40. de Lemos JA, Morrow DA, Sabatine MS, et al. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. *Circulation* 2003; 107: 690–695.
  41. Kervinen H, Manttari M, Kaartinen M, et al. Prognostic usefulness of plasma monocyte/macrophage and T-lymphocyte activation markers in patients with acute coronary syndromes. *Am J Cardiol* 2004; 94: 993–996.
  42. Falcone C, Minoretto P, D'Angelo A, et al. Markers of eosinophilic inflammation and risk prediction in patients with coronary artery disease. *Eur J Clin Invest* 2006; 36: 211–217.
  43. Emanuele E, Falcone C, D'Angelo A, et al. Association of plasma eotaxin levels with the presence and extent of angiographic coronary artery disease. *Atherosclerosis* 2006; 186: 140–145.
  44. Bursill CA, Channon KM, Greaves DR. The role of chemokines in atherosclerosis: recent evidence from experimental models and population genetics. *Curr Opin Lipidol* 2004; 15: 145–149.
  45. Szalai C, Duba J, Prohaszka Z, et al. Involvement of polymorphisms in the chemokine system in the susceptibility for coronary artery disease (CAD). Coincidence of elevated Lp(a) and MCP-1 –2518 G/G genotype in CAD patients. *Atherosclerosis* 2001; 158: 233–239.
  46. Kim MP, Wahl LM, Yanek LR, et al. A monocyte chemoattractant protein-1 gene polymorphism is associated with occult ischemia in a high-risk asymptomatic population. *Atherosclerosis* 2006; epub ahead of print.
  47. Alonso-Villaverde C, Coll B, Parra S, et al. Atherosclerosis in patients infected with HIV is influenced by a mutant monocyte chemoattractant protein-1 allele. *Circulation* 2004; 110: 2204–2209.
  48. Brenner D, Labreuche J, Touboul PJ, et al.; GENIC Investigators. Cytokine polymorphisms associated with carotid intima-media thickness in stroke patients. *Stroke* 2006; 37: 1691–1696.
  49. McDermott DH, Yang Q, Kathiresan S, et al. CCL2 polymorphisms are associated with serum monocyte chemoattractant protein-1 levels and myocardial infarction in the Framingham Heart Study. *Circulation* 2005; 112: 1113–1120.
  50. Ortlepp JR, Vesper K, Mevissen V, et al. Chemokine receptor (CCR2) genotype is associated with myocardial infarction and heart failure in patients under 65 years of age. *J Mol Med* 2003; 81: 363–367.
  51. Gonzalez P, Alvarez R, Batalla A, et al. Genetic variation at the chemokine receptors CCR5/CCR2 in myocardial infarction. *Genes Immun* 2001; 2: 191–195.
  52. Benkirane M, Jin DY, Chun RF, et al. Mechanism of transdominant inhibition of CCR5-mediated HIV-1 infection by ccr5Δ32. *J Biol Chem* 1997; 272: 30603–30606.
  53. Pai JK, Kraft P, Cannuscio CC, et al. Polymorphisms in the CC-chemokine receptor-2 (CCR2) and –5 (CCR5) genes and risk of coronary heart disease among US women. *Atherosclerosis* 2006; 186: 132–139.
  54. Simeoni E, Winkelmann BR, Hoffmann MM, et al. Involvement of polymorphisms in the chemokine system in the susceptibility for coronary artery disease (CAD). Coincidence of elevated Lp(a) and MCP-1 –2518 G/G genotype in CAD patients. *Eur Heart J* 2004; 25: 1438–1446.
  55. Boger CA, Fischeder M, Deinzer M, et al. RANTES gene polymorphisms predict all-cause and cardiac mortality in type 2 diabetes mellitus hemodialysis patients. *Atherosclerosis* 2005; 183: 121–129.
  56. Cybulsky MI, Hegele RA. The fractalkine receptor CX3CR1 is a key mediator of atherogenesis. *J Clin Invest* 2003; 111: 1118–1120.
  57. Moatti D, Faure S, Fumeron F, et al. Polymorphism in the fractalkine receptor CX3CR1 as a genetic risk factor for coronary artery disease. *Blood* 2001; 97: 1925–1928.
  58. McDermott DH, Halcox JP, Schenke WH, et al. Association between polymorphism in the chemokine receptor CX3CR1 and coronary vascular endothelial dysfunction and atherosclerosis. *Circ Res* 2001; 89: 401–407.
  59. McDermott DH, Fong AM, Yang Q, et al. Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. *J Clin Invest* 2003; 111: 1241–1250.
  60. Niessner A, Marculescu R, Haschemi A, et al. Opposite effects of CX3CR1 receptor polymorphisms V249I and T280M on the development of acute coronary syndrome. A possible implication of fractalkine in inflammatory activation. *Thromb Haemost* 2005; 93: 949–954.
  61. Hegele RA. SNP judgments and freedom of association. *Arterioscler Thromb Vasc Biol* 2002; 22: