Clinical Investigation and Reports

Association Between Plasma Levels of Monocyte Chemoattractant Protein-1 and Long-Term Clinical Outcomes in Patients With Acute Coronary Syndromes

James A. de Lemos, MD; David A. Morrow, MD, MPH; Marc S. Sabatine, MD, MPH; Sabina A. Murphy, MPH; C. Michael Gibson, MD, MS; Elliott M. Antman, MD; Carolyn H. McCabe, BS; Christopher P. Cannon, MD; Eugene Braunwald, MD

Background—Monocyte chemoattractant protein-1 (MCP-1) is a chemokine responsible for the recruitment of monocytes to sites of inflammation. MCP-1 appears to play a critical role at multiple stages in atherosclerosis, including the initiation of the fatty streak, promotion of plaque instability, and remodeling after myocardial infarction.

Methods and Results—MCP-1 was measured from frozen plasma specimens in 279 healthy volunteers and 2270 patients with acute coronary syndromes enrolled in the Oral Glycoprotein IIb/IIIa Inhibition with Orbofiban in Patients with Unstable Coronary Syndromes (OPUS-TIMI) 16 trial. Median [25th, 75th percentiles] MCP-1 levels were 157 [124, 196] pg/mL in healthy volunteers and 178 [128, 238] pg/mL in the OPUS-TIMI 16 population (P<0.001). In OPUS-TIMI 16, baseline MCP-1 levels were associated with older age, female sex, hypertension, diabetes, prior coronary disease, and renal insufficiency (P<0.01 for each) but not with smoking status, body mass index, ejection fraction, troponin I or C-reactive protein. After adjustment for differences in baseline characteristics, ECG changes, troponin I, and C-reactive protein, an MCP-1 level >75th percentile (corresponding to the 90th percentile in the healthy volunteers) was associated with an increased risk of death or myocardial infarction through 10 months of follow-up (adjusted hazard ratio, 1.53; 95% CI, 1.09 to 2.14; P=0.01).

Conclusions—In a large cohort of patients with acute coronary syndromes, an elevated baseline level of MCP-1 was associated with traditional risk factors for atherosclerosis as well as an increased risk for death or myocardial infarction, independent of baseline variables. Because it appears to play a crucial role at multiple stages of atherosclerosis, MCP-1 is attractive as a surrogate biomarker and merits further study as a potential therapeutic target. (Circulation. 2003;107:690-695.)

Key Words: infarction ■ ischemia ■ leukocytes ■ inflammation

The infiltration of circulating monocytes into the blood vessel wall, where they are transformed into lipid-laden foam cells, is one of the earliest events in the development of the atherosclerotic plaque. In later stages of atherosclerosis, plaques that are “vulnerable” to rupture are characterized by a predominance of macrophages.1 Here, macrophages appear to secrete a number of proteolytic enzymes that degrade the extracellular matrix of the protective fibrous cap, promoting plaque instability and the subsequent cascade of events leading to the development of unstable angina or acute myocardial infarction (MI).2 After MI, monocytes are rapidly recruited to the infarct zone, where they promote wound healing and turnover of the extracellular matrix, as well as contributing to adverse processes such as reperfusion injury and ventricular remodeling.3 Thus, monocyte/macrophages play a critical role at multiple stages in atherosclerosis—initiation of the fatty streak, promotion of plaque instability, and remodeling after MI.

Monocyte chemoattractant protein-1 (MCP-1) is a chemokine responsible for the recruitment of monocytes to sites of inflammation.4 MCP-1 also recruits monocytes into atherosclerotic lesions and into the infarct zone after MI.5 In animal models, the level of expression of MCP-1 and its monocyte receptor (CCR-2) are directly related to the extent of atherosclerosis and macrophage infiltration into the atherosclerotic lesion.6–9 Recently, several investigators have found higher circulating concentrations of MCP-1 in humans associated with older age,10 hypertension,11 hypercholesterolemia,12 and kidney failure,13 and lower circulating levels associated with estrogen replacement4 and HMG-CoA reductase inhibitor therapy.12

In several small case-control studies, plasma MCP-1 levels were found to be highest among patients with acute coronary

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From the TIMI Study Group (J.A.d.L., D.A.M., M.S.S., S.A.M., C.M.G., E.M.A., C.H.M., C.P.C., E.B.); Donald W. Reynolds Cardiovascular Clinical Research Center, the University of Texas, Southwestern Medical School, Dallas, Tex (J.A.d.L.); the Cardiovascular Division, Brigham and Women’s Hospital, Boston, Mass (D.A.M., M.S.S., E.M.A., C.P.C., E.B.); and Harvard Clinical Research Institute, Boston Mass (S.A.M., C.M.G.).
Correspondence to James A. de Lemos, MD, UT Southwestern Medical Center, 5323 Harry Hines Blvd, Room CS 7.102, Dallas, TX 75390-9047.
E-mail james.delemos@utsouthwestern.edu
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syndromes (ACS), intermediate among patients with stable coronary disease, and lowest among healthy control subjects; however, there was considerable overlap in MCP-1 levels between the groups.\(^{15-17}\) No prospective data are available evaluating the relation between plasma MCP-1 levels and outcomes in patients with ACS.

**Methods**

**Study Population**

The Oral Glycoprotein IIb/IIIa Inhibition with Orbociban in Patients with Unstable Coronary Syndromes (OPUS-TIMI 16) Trial was a randomized, multicenter trial comparing an oral glycoprotein IIb/IIIa inhibitor, orbociban, with placebo in 10,288 patients with ACS. Patients were eligible for enrollment if they presented within 72 hours of the onset of ischemic symptoms and met one of the following criteria: age ≥65 with diabetes or vascular disease; prior coronary disease; dynamic ECG changes; or elevated cardiac markers.\(^{18}\) The study was approved by the institutional review board of each hospital, and all patients provided written informed consent. Patients were randomly assigned to one of the following three treatment arms: 50 mg orbociban twice daily (50/50 group), 50 mg orbociban twice daily for 1 month, followed by 30 mg orbociban twice daily (50/30 group), or placebo. The OPUS-TIMI 16 study was terminated prematurely because increased mortality rates were observed in the 50/30 group. No increase in death or ischemic events was observed in the 50/50 group.\(^{18}\) The present study was conducted in all patients assigned to the 50/50 group who had sufficient plasma remaining to measure MCP-1 (n=2270).

**Blood Sampling**

Details of the blood collection protocol have been previously reported.\(^{19}\) Briefly, plasma samples were collected before the initiation of study medication in citrate anticoagulant and frozen at the study site within 60 minutes of collection. The specimens were subsequently stored at −70°C. B-type natriuretic peptide (BNP) and cardiac troponin I (cTnI) were previously measured in all patients by using assays developed at Biosite, Inc (San Diego, Calif.).\(^{19}\) C-reactive protein (CRP) was previously measured in an 802-patient subset of the substudy population, using a commercially available assay (Dade Behring Inc).

**MCP-1 Assay**

To approximate the normal range for MCP-1, citrate plasma samples were collected from 279 healthy volunteers and stored at −70°C. Assays for MCP-1 were performed in both the healthy volunteers and the OPUS-TIMI 16 cohort with the use of murine anti–MCP-1 antibodies generated at Biosite, using phage display and recombinant protein expression techniques. Recombinant human MCP-1 antigen made by Biosite was used both as the immunogen and as the calibrator for assay standardization. Assays were performed in 384-well microtiter plates on a robotic high-throughput platform (TECAN Genesis RSP 200/8). The concentration of MCP-1 was quantified by detecting the binding of alkaline phosphatase-conjugated antibody. All samples were run in duplicate. The minimal detectable concentration for the assay was 40 pg/mL, and the upper end of the reportable range was 2000 pg/mL. The coefficients of variation at 132 pg/mL, 165 pg/mL, 313 pg/mL, and 621 pg/mL were 15.0%, 10.5%, 4.3%, and 3.5%, respectively.

**Clinical End Points**

All-cause death, nonfatal MI, recurrent ischemia requiring urgent revascularization, congestive heart failure (CHF), and the composite of death or MI were evaluated through the end of the 10-month follow-up period. MI and recurrent ischemia were defined using previously reported criteria\(^{20}\) and adjudicated by a clinical events committee. The end point of new or worsening CHF was not adjudicated.

**Statistical Analyses**

The concentration of MCP-1 was compared between healthy subjects and those enrolled in the OPUS-TIMI 16 study by use of the Wilcoxon rank sum test. Patients from the OPUS-TIMI 16 study were divided into quartiles on the basis of their concentration of MCP-1 at the time of enrollment into the trial. Means and proportions for baseline variables were compared across quartiles by using ANOVA for continuous variables and \(\chi^2\) trend tests for categorical variables.

Cumulative hazard functions were used to estimate the frequency of adverse events at the end of the 10-month follow-up period. The log-rank test was used to compare outcomes across quartiles and between patients with MCP-1 levels above versus equal to or below the 75th percentile. The selection of a dichotomous threshold at the 75th percentile was retrospectively defined. For the end point of death or MI, a Cox proportional hazards model was used to adjust for differences in baseline characteristics between patients with and without elevation (>75th percentile) in MCP-1. The following variables were included in the model: age; history of diabetes, hypertension, hypercholesterolemia, or prior coronary disease; index diagnosis (unstable angina, non–ST-elevation MI, ST-elevation MI); creatinine clearance; ST-segment deviation >1 mm on the presenting ECG; elevated cTnI (>0.1 µg/L), and elevated CRP (>1.5 µg/L). Because CRP data were available in only a subset of the population, the model was run with and without inclusion of CRP.

**Results**

In a population of 279 healthy volunteers (mean age, 34±10 years, 50% male) the median MCP-1 concentration was 157 pg/mL, and the 25th, 75th, 90th, and 95th percentiles were 124, 196, 240, and 274 pg/mL, respectively. The study population consisted of 2270 patients, with a median MCP-1 level of 178 pg/mL and 25th, 75th, 90th, and 95th percentiles of 128, 238, 325, and 392 pg/mL, respectively (\(P<0.0001\) versus normal population).

**Association With Baseline Clinical Variables and Other Biomarkers**

MCP-1 levels were positively associated with older age, female sex, hypertension, diabetes, prior coronary artery disease, history or physical examination evidence of CHF, renal insufficiency, and elevated levels of BNP. In contrast, there was no association with smoking status, body mass index, ejection fraction, cTnI, or CRP (n=802 for CRP). There was also no association between the concentration of MCP-1 and the time from symptom onset to enrollment in the trial (Table 1).

**Association With Clinical Outcomes**

Rates of death and the composite of death or MI though 10 months were similar among patients in the first three quartiles of MCP-1 levels (Figure 1). When compared with the first three quartiles, patients in the fourth quartile tended to be more likely to die (HR, 1.43; 95% CI, 0.89 to 2.31; \(P=0.13\)), and were more likely to suffer death or MI (HR, 1.69; 95% CI, 1.24 to 2.31; \(P=0.001\)) (Figure 2). In contrast, there was no significant association between elevated MCP-1 levels and recurrent ischemia or CHF (Table 2).

After adjustment for age; history of diabetes, hypertension, hypercholesterolemia, or coronary disease; creatinine clearance; ST deviation; and cTnI; an elevated level of MCP-1 (>75th percentile) remained associated with an increased risk of death or MI (n=2127; adjusted HR, 1.42; 95% CI, 1.02 to
After further adjustment for elevation in CRP (in the subset of patients with available CRP data), MCP-1 elevation was associated with an increased risk for death or MI (n=748; adjusted HR, 1.53; 95% CI, 1.09 to 2.14; P=0.01).

**Discussion**

In a large contemporary cohort of patients with ACS, a plasma concentration of MCP-1 in the fourth quartile (>238 pg/mL), corresponding to approximately the 90th percentile in the normal population, was associated with a significant increase in the risk for death or MI over 10 months of follow-up, after adjustment for traditional risk factors, ECG changes, and levels of cTnl and CRP. In contrast to previous small case-control studies,15–17 the present study population was heterogeneous and included patients treated with both invasive and conservative strategies.

Significant associations were observed between plasma levels of MCP-1 and other risk factors for coronary atherosclerosis, such as age, hypertension, diabetes, renal insufficiency, and vascular disease, confirming results from prior studies in subjects without ACS.10–13 Despite these associations, there was only modest difference in the distribution of MCP-1 between the normal population and the OPUS-TIMI 16 population. This may explain the observation that prognosis was similar between patients in quartiles 1 to 3 but significantly worse in patients in the 4th quartile, who had levels of MCP-1 distinct from the “normal range.”
Pathophysiological Implications

The close association between MCP-1 and other markers of atherosclerosis noted in this and prior studies provides additional support for a direct pathogenic role of MCP-1 in the initiation and progression of coronary atherosclerosis in humans. MCP-1 is produced by all cell types present in atheroma, although the macrophage and endothelial cell appear to be the primary sources. Expression of both MCP-1 and its monocyte receptor are upregulated in hypercholesterolemia, a finding that appears to be mediated directly by lipids and lipid metabolites. LDL receptor–deficient mice that undergo targeted deletion of the MCP-1 gene are resistant to the effects of a cholesterol-rich diet and have markedly less atherosclerosis than similar mice who do not undergo deletion of the MCP-1 gene. Similarly, mice lacking the receptor for MCP-1 (CCR2) are resistant to the development of atherosclerosis.

The progression from a vulnerable plaque to plaque rupture and to the ACS phenotype may also be influenced by MCP-1. Activated platelets and thrombin, which are universally present in ACS, have been shown to induce endothelial expression of MCP-1. In addition to its chemoattractant properties, MCP-1 appears to induce tissue factor production, a feature by which it might promote thrombosis in the setting of plaque rupture.

MCP-1 may also contribute to complications after MI, including acute reperfusion injury and chronic ventricular remodeling and heart failure. Within 1 hour after ischemia-reperfusion, MCP-1 gene expression is increased in the infarct territory. In addition to recruiting monocytes, MCP-1 increases endothelial cell expression of ICAM-1, which may contribute to neutrophil-mediated reperfusion injury.

Clinical Implications

Although the present study suggests that MCP-1 may be useful for risk prediction after ACS, these findings are preliminary and require confirmation in additional studies. Although statistically different, the distribution of MCP-1 values in the normal population and the study population overlapped considerably, indicating that MCP-1 will not be useful for diagnosing ACS. However, given the wealth of scientific data implicating MCP-1 early in the development of atherosclerosis and the close associations observed between MCP-1 Levels >75th Percentile and Those With Levels ≤75th Percentile

TABLE 2. Comparison of Outcomes Between Patients With MCP-1 Levels >75th Percentile and Those With Levels ≤75th Percentile

<table>
<thead>
<tr>
<th>End Point, %</th>
<th>MCP-1 ≤238 pg/mL (n=1703)</th>
<th>MCP-1 &gt;238 pg/mL (n=567)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>3.7</td>
<td>7.0</td>
<td>0.13</td>
</tr>
<tr>
<td>MI</td>
<td>5.1</td>
<td>9.8</td>
<td>0.004</td>
</tr>
<tr>
<td>Death or MI</td>
<td>8.0</td>
<td>15.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urgent revascularization</td>
<td>6.5</td>
<td>6.2</td>
<td>0.88</td>
</tr>
<tr>
<td>CHF</td>
<td>2.7</td>
<td>3.4</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Event rates are based on 10-month Kaplan-Meier survival estimates.
levels of MCP-1 and traditional risk factors, future studies should also evaluate the potential role of MCP-1 in identifying atherosclerosis earlier in its natural history. Importantly, a number of preventive interventions have been shown to reduce either tissue expression or circulating levels of MCP-1, including treatment with statins, thiazolidinediones, hormone replacement, and even consumption of red wine. In summary, MCP-1 is associated with traditional risk factors for atherosclerosis, but the prognostic value of this marker appears to be independent of these risk factors; furthermore, MCP-1 levels appear to be modifiable with currently available preventive therapies. These observations suggest that MCP-1 may eventually prove to be useful as a surrogate biomarker in patients with or at risk for atherosclerosis and its complications.

**Limitations**

Although this study represents the first large-scale evaluation of MCP-1 as a cardiac biomarker, it has a number of important limitations. At low concentrations, orbofib may have partial agonist actions at the GP IIb/IIIa receptor and may be proinflammatory. Although blood samples were collected before the administration of orbofib, we cannot exclude the possibility of an interaction between baseline levels of MCP-1, orbofib, and subsequent events, because we did not measure MCP-1 levels in the placebo arm of the trial. Lipid measurements were not performed. However, prior history of hyperlipidemia and lipid-lowering therapy were not associated with MCP-1 levels. Serial measurements of MCP-1 were not performed, so it is not possible to determine the optimal time for measuring this cytokine. No association was observed between time to randomization and MCP-1 levels, however.

The normal population was small and was not age-matched with the study population. Clearly, larger studies in healthy populations are needed to determine the normal range of MCP-1 and the factors that influence MCP-1 levels in the absence of atherosclerotic disease. In the present study, the association between MCP-1 and the occurrence of death or MI was independent of age. Finally, although a quartiles analysis was prospectively specified, no dichotomous threshold was prespecified. The threshold at the 75th percentile of the study population (and the 90th percentile of the normal population) was determined post hoc and will require prospective validation.

**Conclusions**

In a large group of patients with ACS, a level of MCP-1 above the 75th percentile was associated with traditional risk factors for atherosclerosis as well as an increased risk for death or MI, independent of baseline variables. Because it appears to play a crucial role at multiple stages of atherosclerosis, MCP-1 is attractive as a surrogate biomarker and merits further study as a potential therapeutic target. These observations should be considered exploratory and require independent validation in prospective cohorts.

**Acknowledgments**

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**References**


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