Osteopontin plasma levels and accelerated atherosclerosis in patients with CAD undergoing PCI: a prospective clinical study

Annmaria Mazzone\textsuperscript{a}, Maria Serena Parri\textsuperscript{a}, Daniela Giannessi\textsuperscript{b}, Marcello Ravani\textsuperscript{a}, Marco Vaghetto\textsuperscript{a}, Paola Altieri\textsuperscript{c}, Laura Casalino\textsuperscript{c}, Mariastella Maltinti\textsuperscript{b}, Manrico Balbi\textsuperscript{c}, Antonio Barsotti\textsuperscript{c} and Sergio Berti\textsuperscript{a}

Objectives Growing evidence supports the role played by inflammation in atherosclerosis. Identifying sensitive biomarkers is useful in predicting accelerated atherosclerosis. We investigated prospectively the relationship between plasma levels of inflammatory biomarkers [osteopontin, C-reactive protein (CRP), interleukin-6 (IL-6)] and instant restenosis, and rapid coronary plaque progression in patients with coronary artery disease (CAD) undergoing percutaneous coronary intervention (PCI).

Methods We studied 77 patients with CAD: 45 affected by unstable angina/non-ST elevation myocardial infarction [acute coronary syndrome (ACS)], and 32 by chronic coronary syndrome (CCS). Plasma osteopontin, IL-6, and CRP levels were measured before intervention in all patients; measurements were carried out on the basis of the following time course at 1, 15, 30, 90, and 180 days follow-up in a subgroup of 39 consenting patients. Clinical and biohumoral data were correlated with baseline and 6-month PCI follow-up angiography.

Results Osteopontin, IL-6, and CRP were higher in patients with ACS than in those with CCS (analysis of variance: \(P<0.001, 0.05, \) and 0.05, respectively). Baseline osteopontin levels proved to be associated with rapid coronary plaque progression (\(P=0.005\)) and instant restenosis (\(P=0.05\)). The highest osteopontin levels were found in patients with CAD with both rapid plaque progression and instant restenosis (\(P=0.003\)). PCI increased inflammatory markers acutely, and osteopontin remained elevated in patients with ACS. Patients with ACS showed a higher percentage (74%) of rapid plaque progression than those with CCS (26%) (\(P<0.05\)).

Conclusion The study prospectively shows the link between inflammatory status and accelerated atherosclerosis in patients with CAD undergoing PCI. The baseline and persistent rise of osteopontin is an expression of its contribution to the accelerated plaque progression, and therefore, osteopontin may be a useful prognostic biomarker. Coron Artery Dis 00:000–000 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Coron Artery Disease 2011, 00:000–000

Keywords: accelerated atherosclerosis, coronary artery disease inflammation, instant restenosis, osteopontin

\textsuperscript{a}Heart Hospital, Fondazione Toscana G. Monasterio/CNR, Massa, \textsuperscript{b}CNR Institute of Clinical Physiology, CNR Research Area, Pisa and \textsuperscript{c}Department of Internal Medicine, Cardiology Unit, University of Genova, Italy

Correspondence to Annmaria Mazzone, MD, FESC, Department of Cardiology, Heart Hospital G. Monasterio Foundation, CNR, Via Aurelia Sud Montepepe 54100, Massa, Italy
Tel: +39 0585 493 638; fax: +39 0585 493 601; e-mail: mazzone@ifc.cnr.it

Received 2 December 2010 Accepted 26 December 2010

Introduction

Inflammatory pathways, supported by immune-system mediators, play a pivotal role in all stages of atherosclerosis, conditioning the clinical outcomes [1–3]. Higher chronic vascular inflammatory response is involved in the development of restenosis after balloon angioplasty stent implantation [4], and accelerated atherosclerosis has also been found to prevail in patients with immunologic diseases, rheumatoid arthritis, and diabetes [2,5]. Moreover, acute coronary syndrome (ACS) is characterized by the activation, beyond the culprit lesion, of multiple plaques in the coronary tree and in the systemic arterial bed [6,7]. The widespread inflammatory response, which is further increased by the primary percutaneous coronary intervention (PCI) procedure, affects the biological and proliferative processes in the coronary tree, leading to instant restenosis and/or rapid progression of nonculprit untreated plaques [4,8,9]. ‘De novo stenosis’ and ‘instant restenosis’ are indicative of accelerated atherosclerosis [10]. The rapid progression of coronary artery disease (CAD), either silent or with acute events [11], seems to be a powerful predictor of cardiovascular risk [1] and is associated with increased levels of C-reactive protein (CRP), markers of endothelial and macrophage activation, and multiple coronary complex stenosis [8,9]. The evidence of sensitive inflammatory biomarkers with a predictive value for restenosis and cardiovascular outcomes after PCI may be useful in the screening and treatment of patients with a higher likelihood of accelerated atherosclerosis.
Osteopontin, a secreted calcium-binding glycoprophosphoprotein involved in bone remodeling, tumor progression, inflammation, and cell-mediated immunity, plays an important role in cardiovascular disease and atherosclerosis [12,13]. Osteopontin mRNA and protein have been detected both in human primary stable/unstable plaque and in the hyperplastic neointima of instant restenosis [12–14]. Increased osteopontin plasma levels were found to be correlated with the extent of CAD and the future adverse cardiac events in stable angina [15,16].

We prospectively assessed whether the magnitude degree of systemic inflammatory status is associated with instant restenosis and rapid plaque progression in untreated vessels in patients with CAD undergoing PCI by quantitative coronary angiography at 6-month follow-up, and which plasma inflammatory markers, among interleukin-6 (IL-6), CRP, and osteopontin, are more closely associated with accelerated atherosclerosis.

Materials and methods

Patients

We studied 77 consecutive patients with CAD (66 men, mean age: 61 ± 10 years), undergoing PCI: 45 with ACS (unstable angina or non-ST elevation myocardial infarction (NSTEMI) and 32 with chronic coronary syndrome (CCS)).

Unstable angina was diagnosed in patients presenting with chest pain at rest with myocardial ischemia within the preceding 12 h, and transient ST segment depression or T-wave inversion, or both, in two or more contiguous electrocardiographic leads; a diagnosis of NSTEMI was made on the basis of a history consistent with unstable angina and a circulating troponin T concentration of greater than or equal to 1 coronary artery (Table 1).

Exclusion criteria were myocardial infarction with ST-segment elevation or Q waves on the electrocardiogram; a diagnosis of NSTEMI was made on the basis of a history consistent with unstable angina and a circulating troponin T concentration of greater than or equal to 1 coronary artery (Table 1).

Materials and methods

Clinical follow-up

At 1–6 months after PCI, patients underwent a clinical follow-up that included a stress test designed to detect any major adverse cardiac events (cardiac death, myocardial infarction, recurrent angina, and ischemia-driven target revascularization). Angiographic follow-up was scheduled for all patients 6 months after PCI (median: 6.8 months, maximum: 8.8 months).

Laboratory methods

In patients with ACS, the baseline blood samples were drawn after admission to the emergency department and before angiography in patients with stable angina. A consenting subgroup of 39 patients agreed to take part in the follow-up, and to provide blood samples 1, 14, 30, 60, 90, and 180 days after PCI to evaluate the baseline and prospective time course of systemic inflammatory response by osteopontin, IL-6, and CRP. Blood was drawn into pyrogen-free test tubes containing EDTA as the anticoagulant and aprotinin. The tubes were immersed in melting ice and centrifuged (1000 g at 4°C for 15 min) within 15 min of sampling. Multiple aliquots of serum and plasma were stored at −80°C until analysis. Osteopontin was quantified by a commercially available enzyme-linked immunosorbeny assay kit (Human OPN Titer Zyme EIA, BioVendor-Laboratorie, Heidelberg, Germany). We performed the same measurements at scheduled follow-up visits 6 months after PCI (median: 6.8 months, maximum: 8.8 months).

HGM-CoA reductase inhibitors.

Aspirin, ticlopidine, or clopidogrel for 90 days after PCI and aspirin (100 mg/daily) indefinitely.

HGM-CoA reductase inhibitors.

Aspirin, ticlopidine, or clopidogrel for 90 days after PCI and aspirin (100 mg/daily) indefinitely.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>ACS (n=45)</th>
<th>CCS (n=32)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>63 ± 10</td>
<td>65 ± 10</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>37 (82%)</td>
<td>28 (87%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>34 (76%)</td>
<td>27 (84%)</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>129 ± 20</td>
<td>132 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>38 (84%)</td>
<td>20 (62%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>195 ± 44</td>
<td>186 ± 38</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>124 ± 43</td>
<td>115 ± 33</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>44 ± 12</td>
<td>42 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Osteopontin basal level (ng/ml)</td>
<td>971 ± 471</td>
<td>566 ± 296</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP basal level (mg/dl)</td>
<td>7.3 ± 18.4</td>
<td>1.7 ± 3.0</td>
<td>0.033</td>
</tr>
<tr>
<td>IL-6 basal level (pg/ml)</td>
<td>4.0 ± 3.9</td>
<td>1.6 ± 1.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>12 (27%)</td>
<td>9 (28%)</td>
<td>NS</td>
</tr>
<tr>
<td>Current smoking</td>
<td>30 (67%)</td>
<td>25 (78%)</td>
<td>NS</td>
</tr>
<tr>
<td>Earlier infarction</td>
<td>10 (22%)</td>
<td>11 (34%)</td>
<td>NS</td>
</tr>
<tr>
<td>Instant restenosis</td>
<td>11 (24%)</td>
<td>3 (9%)</td>
<td>NS</td>
</tr>
<tr>
<td>ATS progression</td>
<td>23 (51%)</td>
<td>8 (29%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Pharmacologic treatment (n, %)

Aspirin, ticlopidine, or clopidogrel for 90 days after PCI and aspirin (100 mg/daily) indefinitely.

HGM-CoA reductase inhibitors.

Aspirin, ticlopidine, or clopidogrel for 90 days after PCI and aspirin (100 mg/daily) indefinitely.

Coronary artery disease

LVEF (%) | 52 ± 9 | 55 ± 8 | NS
| 1-vessel disease | 28 (62%) | 14 (44%) | NS
| 2-vessels disease | 12 (27%) | 15 (47%) | NS
| 3-vessels disease | 4 (9%) | 3 (9%) | NS
| Dilated vessels LAD artery | 17 (38%) | 4 (12%) | NS
| LCx artery | 5 (11%) | 1 (3%) | NS
| RCA | 5 (11%) | 2 (6%) | NS
| Small vessels | 4 (9%) | 0 (0%) | NS

ACS, acute coronary syndrome; ATS, atherosclerosis; CCS, chronic coronary syndrome; CRP, C-reactive protein; HDL, high-density lipoprotein; HGM-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IL-6, interleukin-6; LAD, left anterior descending artery; LCx, left circumflex artery; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction; NS, not significant; RCA, right coronary artery; SBP, systolic blood pressure.

At 1–6 months after PCI, patients underwent a clinical follow-up that included a stress test designed to detect any major adverse cardiac events (cardiac death, myocardial infarction, recurrent angina, and ischemia-driven target revascularization). Angiographic follow-up was scheduled for all patients 6 months after PCI (median: 6.8 months, maximum: 8.8 months).

Laboratory methods

In patients with ACS, the baseline blood samples were drawn after admission to the emergency department and before angiography in patients with stable angina. A consenting subgroup of 39 patients agreed to take part in the follow-up, and to provide blood samples 1, 14, 30, 90, and 180 days after PCI to evaluate the baseline and prospective time course of systemic inflammatory response by osteopontin, IL-6, and CRP. Blood was drawn into pyrogen-free test tubes containing EDTA as the anticoagulant and aprotinin. The tubes were immersed in melting ice and centrifuged (1000 g at 4°C for 15 min) within 15 min of sampling. Multiple aliquots of serum and plasma were stored at −80°C until analysis. Osteopontin was quantified by a commercially available enzyme-linked immunosorbeny assay kit (Human OPN Titer Zyme EIA, BioVendor-Laboratorie, Heidelberg, Germany). We performed the same measurements at scheduled follow-up visits 6 months after PCI (median: 6.8 months, maximum: 8.8 months).
Osteopontin: a prognostic marker in ATS Mazzone et al. 3

Statistical analysis
Results for normally distributed continuous variables are expressed as mean ± standard deviation or standard error of the mean, whereas continuous variables with non-normal distribution are presented as median values and interquartile intervals; categorical data variables are expressed as percentages. Categorical variables and proportions were compared by χ² analysis or Fisher’s exact test when required. Continuous variables were compared by Student’s t-test. Group D differences between patients with or without instent restenosis, and with or without rapid CAD plaque progression, were assessed by χ² analysis and Student’s t-test for categorical variables and continuous variables, respectively. Multiple comparisons were assessed by unpaired two-tailed t-test of log-transformed values (analysis of variance), followed by the post-hoc Bonferroni correction. Analysis of variance for repeated measures followed by a multiple comparison test (Bonferroni test) was carried out to evaluate the significance of changes in osteopontin, CRP, and IL-6 values. Correlations were determined by Pearson’s correlation test.

A P value of less than 0.05 (two-tailed) was considered statistically significant. The SPSS statistical software package (SPSS Inc., Chicago, Illinois, USA) was used for all calculations.

Results
We subdivided the patients with CAD into two groups: ACS (unstable angina/NSTEMI) and CCS. The baseline clinical biochemical and angiographical data are illustrated in Table 1.

Clinical data
No significant differences in cardiovascular risk factors or in pharmacological treatment were found between the two groups, and furthermore, no fatal events had occurred at 6-month follow-up (overall mortality rate was 0%). Twelve of the patients were referred for atypical chest pain, 30% complained of typical chest pain, and 58% remained asymptomatic. Two patients with ACS were admitted to the Hospital for ACS related to instent restenosis, while no cases of heart failure was reported. Six months after PCI, all patients underwent a treadmill test (positive in 10%, negative in 33%, and nondiagnostic for ischemia in 57%).

Biohumoral data
All the baseline inflammatory biomarkers (osteopontin, IL-6, CRP) were higher in patients with ACS than in those with CCS. Osteopontin levels were significantly increased in patients with ACS compared with those with CCS (P < 0.0001) and healthy controls (P < 0.0001). IL-6 levels were more elevated in patients with ACS compared with those with CCS (P = 0.024) and controls (P = 0.002). CRP levels were higher in patients with ACS

Assay Designs, Inc., Ann Arbor, Michigan, USA) (sensitivity: 4.9 ± 0.30 ng/ml, variability: 50 ± 32, coefficient of variation: 17.9%). IL-6 was measured by a commercially available high-sensitivity enzyme-linked immunosorbent assay kit (Dialclone Research, Besancon, France) (sensitivity: 0.53 ± 0.12 pg/ml, variability: 21.1 ± 0.64, coefficient of variation: 8.7%). CRP was measured by a turbidimetric assay (Synchroom CXR/instrument, Beckman Coulter, Inc., Fullerton, California, USA) (sensitivity: 0.5 mg/dl, range of concentration: 0.5–2.0 mg/dl).

In patients with ACS (unstable angina/NSTEMI), PCI and stenting were performed within 72 h of admission, according to standard techniques [17]. The first coronary angiograms were obtained before/after PCI, and angiographic follow-up was scheduled for all patients 6 months after PCI (median: 6.8 months, maximum: 8.8 months).

The angiograms were obtained in routine standard projections using the Digital Integris 3000 System (Philips, Chicago, Illinois, USA). Coronary stenoses were quantitatively assessed by the automatic edge-detection algorithm (MEDIS, The Netherlands). The angiograms were assessed by three blinded, experienced/independent cardiologists. Paired angiograms were analyzed in different sessions, though not in succession. Intraobserver–interobserver variability for changes in stenosis diameter between angiograms was calculated at 3.5 ± 1.7% and 4.0 ± 2.1%, respectively. Differences in stenosis diameter between the second angiography (immediately after the procedure) and the third angiography (after 6 months) were assessed in 117 stenoses. A stenosis with a diameter reduction of more than 50% was considered hemodynamically significant, and a lesion of less than 50% was considered mild. Measurements of each section were carried out on end-diastolic frames in which the severity of the stenosis seemed maximal. Percent diameter reduction of a coronary stenosis was calculated by comparing the minimal stenosis diameter with the diameter of the reference segment (an angiographically normal segment proximal to the lesion), measured in millimeters. The following formula was used: percent diameter reduction [(diameter or reference segment diameter of stenosis)/(diameter of reference segment)] × 100.

As in an earlier study by Zouridakis et al. [8], rapid CAD progression was diagnosed in the presence of any of the following conditions: (i) a diameter reduction of 50% or more in a previously successfully dilated lesion (stenot and 5 mm proximal and distal) (restenosis); (ii) a diameter reduction of 10% or more of a pre-existing stenosis 50% or more; (iii) a diameter reduction of 30% or more of a stenosis less than 50%; and (iv) development of a new stenosis ( ≥ 30% diameter reduction) in a segment that was normal at the first angiogram, and progression of any lesion to total occlusion at the second angiogram.
compared with those with CCS ($P = 0.033$) and controls ($P = 0.004$) (Tables 1 and 2).

Baseline osteopontin was directly related to the age of the patient ($P < 0.0001$), to CRP ($P < 0.0001$), and to troponin I ($P = 0.0003$).

Higher baseline levels of osteopontin were found in patients with ACS with diabetes than without diabetes (osteopontin $P = 0.005$) and compared with patients with CCS with and without diabetes (Fig. 1).

At angiographic follow-up at 6 months, patients with CAD with higher baseline osteopontin levels showed a rapid coronary plaque progression in untreated vessels ($P = 0.005$), and instant restenosis ($P = 0.05$), compared with nonprogressing patients. CRP and IL-6 levels also increased, but did not differ significantly when the two patient groups were compared (two-tailed t-test) (Table 3, Fig. 2).

The highest baseline osteopontin levels were observed in the patients with ACS who showed both rapid de-novo plaque progression and instant restenosis 6 months after PCI ($P = 0.003$), or rapid CAD progression alone ($P = 0.02$), compared with patients with CCS and nonprogressing patients (Fig. 3).

No risk factors or other biohumorlal parameters were associated with accelerated atherosclerosis. Only the low high-density lipoprotein plasma levels were related to rapid CAD plaque progression ($P = 0.05$) (Table 3).

The time course measurement of plasma inflammatory markers after PCI over 6 months of follow-up was taken in a subgroup of 39 consenting patients. The inflammatory markers increased 24 h after PCI in all patients with CAD, while osteopontin levels continued to be significantly more elevated in patients with ACS than in those with CCS for 6 months (Fig. 4).

### Coronary angiography data

Fifty percent of studied patients had single-vessel disease, 41% had two-vessel disease, and 9% had three-vessel disease. A total of 117 coronary atheromasic lesions were detected, and 91 lesions (77%) were diagnosed as critical and were treated by percutaneous transluminal coronary angioplasty/stenting (mean 1.1 per patient).

The use of drug eluting stents was quite similar in the two groups: 55% in the ACS group and 50% in the CCS group ($P = $ not significant).

Quantitative coronary angiography was used in cases of restenosis and de-novo coronary plaque progression after 6-month coronary angiography follow-up. Instant restenosis was observed in 14 patients with CAD who had been revascularized (19%), and 10 (71%) of them were affected by ACS. The rapid coronary plaque progression of the untreated lesions, at 6 months after PCI, was observed in patients with CAD (40%): prevailing

### Table 2  Baseline inflammatory biomarkers (osteopontin, C-reactive protein, interleukin-6) in coronary artery disease patients and control group

<table>
<thead>
<tr>
<th></th>
<th>ACS ($n=45$)</th>
<th>CCS ($n=32$)</th>
<th>HS (16)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPN (ng/ml)</td>
<td>971±471*</td>
<td>566±296</td>
<td>455±71</td>
<td>&lt;0.0003*</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>7.3±18.4*</td>
<td>1.7±3.0</td>
<td>0.2±0.1</td>
<td>NS*</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.0±3.9*</td>
<td>1.6±1.4</td>
<td>0.3±0.1</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; CAD, coronary artery disease; CCS, chronic coronary syndrome; CRP, C-reactive protein; HS, healthy individuals or controls; IL-6, interleukin-6; NS, not significant; OPN, osteopontin.

* $P<0.05$.
The pathological mechanisms responsible for ACS are believed to involve an acute inflammatory stimulus that contributes to coronary plaque disruption, and studies have shown dense inflammatory cell infiltrate, including macrophages at the site of plaque rupture [3,6,7]. Osteopontin in the plaque is believed to exert its effect through upregulation within, and in proximity to, activated cells, thereby becoming responsible for changes leading to instability, and inducing matrix metalloproteinase release, angiogenesis, hemorrhage, fibrous cap degradation, and thrombotic complications [13,14]. The higher plasma levels of osteopontin in ACS seem to be an indicator of an active inflammation of the unstable coronary plaque and a local/systemic immunomediated response by activated T-lymphocytes [3,6,7,14,18,20,21].

Osteopontin, which is also known as early T-lymphocyte activation 1, acts as a cytokine, promoting adhesion of human blood T-cells, and enhancing Th1 and inhibiting Th2 cytokine expression [13]. Earlier studies have shown that plasma osteopontin levels are elevated in patients with unstable angina and NSTEMI and that this is accompanied by an increase in the number of osteopontin-produced circulating CD4+ T-cells. This supports the hypothesis that through osteopontin, circulating CD4+ T-cells may play a role in the pathophysiology of unstable angina [18,19].

In our study, baseline osteopontin was related to the age of the patients and to troponin-I and CRP, but unlike in an earlier study by Ohmori et al. [15], no correlation was found between the extent of CAD and cardiovascular risk factors. Moreover, osteopontin and CRP levels proved to be significantly higher in the group of patients with ACS affected by type 2 diabetes compared with non-diabetic patients with rapid CAD progression also showed a higher percentage of instant restenosis (35%) than patients without it (6%) (P = 0.001) (Table 3). No significant relationship was found among the other angiographical parameters, such as the number of diseased vessels, or coronary lesion extension, and rapid CAD progression (Table 3).

**Discussion**

This prospective clinical study shows intriguing data on the association between the extent of systemic inflammation and accelerated atherosclerosis. We show how in patients with CAD, preprocedural osteopontin plasma levels, which are more increased in ACS (unstable angina/NSTEMI) than in CCS, are associated with de-novo coronary plaque progression and instant restenosis, at 6-month angiography after PCI.

Our findings are in agreement with earlier data on the increasing systemic inflammatory response (CRP, IL-6, osteopontin) in ACS compared with CCS, as an expression of a more local and widespread acute inflammatory status [7–9,19] (Table 1).

Table 3 Demographical, clinical, biohumoral, and coronary data of patients with and without coronary plaque progression and with and without instant restenosis

<table>
<thead>
<tr>
<th></th>
<th>No progression</th>
<th>Progression</th>
<th>P value</th>
<th>No restenosis</th>
<th>Restenosis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 46)</td>
<td>(n = 31)</td>
<td></td>
<td>(n = 63)</td>
<td>(n = 14)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 ± 11</td>
<td>63 ± 9</td>
<td>NS</td>
<td>64 ± 10</td>
<td>64 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>36 (78%)</td>
<td>29 (94%)</td>
<td>NS</td>
<td>51 (84%)</td>
<td>12 (86%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>36 (78%)</td>
<td>25 (81%)</td>
<td>NS</td>
<td>47 (77%)</td>
<td>11 (77%)</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>130 ± 16</td>
<td>131 ± 21</td>
<td>NS</td>
<td>131 ± 18</td>
<td>127 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>35 (72%)</td>
<td>25 (78%)</td>
<td>NS</td>
<td>46 (75%)</td>
<td>11 (75%)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>195 ± 42</td>
<td>186 ± 41</td>
<td>NS</td>
<td>193 ± 42</td>
<td>189 ± 56</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>122 ± 42</td>
<td>118 ± 35</td>
<td>NS</td>
<td>122 ± 41</td>
<td>114 ± 31</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>45 ± 12</td>
<td>40 ± 9</td>
<td>0.05</td>
<td>43 ± 11</td>
<td>42 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>11 (24%)</td>
<td>10 (32%)</td>
<td>NS</td>
<td>17 (28%)</td>
<td>4 (29%)</td>
<td>NS</td>
</tr>
<tr>
<td>Current smoking</td>
<td>31 (67%)</td>
<td>24 (77%)</td>
<td>NS</td>
<td>44 (72%)</td>
<td>10 (71%)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline OPN (mg/ml)</td>
<td>684 ± 285</td>
<td>977 ± 586</td>
<td>0.005</td>
<td>707 ± 373</td>
<td>1019 ± 666</td>
<td>0.02</td>
</tr>
<tr>
<td>Baseline CRP (mg/dl)</td>
<td>6.5 ± 19.2</td>
<td>3.4 ± 4.6</td>
<td>NS</td>
<td>3.3 ± 4.5</td>
<td>4.3 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline IL-6 (pg/ml)</td>
<td>2.2 ± 2.1</td>
<td>3.7 ± 4.0</td>
<td>NS</td>
<td>2.9 ± 3.3</td>
<td>3.3 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Earlier myocardial infarction</td>
<td>12 (26%)</td>
<td>9 (29%)</td>
<td>NS</td>
<td>20 (33%)</td>
<td>4 (29%)</td>
<td>NS</td>
</tr>
<tr>
<td>ACS</td>
<td>22 (48%)</td>
<td>23 (74%)</td>
<td>0.02</td>
<td>32 (52%)</td>
<td>11 (76%)</td>
<td>NS</td>
</tr>
<tr>
<td>CCS</td>
<td>24 (52%)</td>
<td>8 (28%)</td>
<td>0.02</td>
<td>29 (49%)</td>
<td>3 (29%)</td>
<td>NS</td>
</tr>
<tr>
<td>Instent restenosis</td>
<td>3 (6%)</td>
<td>11 (35%)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-vessel disease</td>
<td>25 (56%)</td>
<td>15 (50%)</td>
<td>NS</td>
<td>31 (49%)</td>
<td>9 (64%)</td>
<td>NS</td>
</tr>
<tr>
<td>2-vessels disease</td>
<td>14 (31%)</td>
<td>13 (43%)</td>
<td>NS</td>
<td>22 (35%)</td>
<td>4 (27%)</td>
<td>NS</td>
</tr>
<tr>
<td>3-vessels disease</td>
<td>5 (11%)</td>
<td>2 (7%)</td>
<td>NS</td>
<td>7 (11%)</td>
<td>1 (7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Small vessels</td>
<td>6 (13%)</td>
<td>1 (3%)</td>
<td>NS</td>
<td>4 (6%)</td>
<td>0 (0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Pharmacologic treatment (n, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>41 (91%)</td>
<td>30 (100%)</td>
<td>NS</td>
<td>58 (92%)</td>
<td>14 (100%)</td>
<td>NS</td>
</tr>
<tr>
<td>Ticlopidin</td>
<td>7 (16%)</td>
<td>6 (17%)</td>
<td>NS</td>
<td>12 (20%)</td>
<td>0 (0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>38 (84%)</td>
<td>25 (83%)</td>
<td>NS</td>
<td>50 (82%)</td>
<td>13 (93%)</td>
<td>NS</td>
</tr>
<tr>
<td>HGM-CoA reductase inhibitors</td>
<td>39 (87%)</td>
<td>25 (83%)</td>
<td>NS</td>
<td>51 (84%)</td>
<td>12 (86%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; CCS, chronic coronary syndrome; CRP, C-reactive protein; HDL, high-density lipoprotein; HGM-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IL-6, interleukin-6; LDL, low-density lipoprotein; OPN, osteopontin; NS, not significant; SBP, systolic blood pressure.
patients and those with CCS (Fig. 1). Although these data were detected in a small sample, they are intriguing because increasing evidence has shown that diabetes is associated with an enhanced inflammatory status, and that inflammatory cells contribute to accelerated plaque progression [5]. Although some investigators earlier found CRP to be a prognostic marker of adverse in-hospital cardiac events, death, acute myocardial infarction, and late instent restenosis in patients with ACS, others reported that the degree of preprocedural inflammatory markers, such as CRP, IL-6, and serum amyloid A, was not a prognostic factor for restenosis [22–25]. In our prospective study, the association between the preprocedural osteopontin levels of patients with CAD undergoing PCI, and coronary plaque progression and instent restenosis at angiographic follow-up may indicate the biomarker’s ability to predict the accelerated atherosclerotic process (Fig. 2). Osteopontin is a modified extracellular matrix glycoprophosphoprotein, which is higher in animal/human primary lesions and in restenotic lesions [13]. Increased osteopontin mRNA was shown in neointimal smooth muscle cells after arterial injury in animal models. Liaw et al. [26] showed that antiosteopontin antibody treatment reduces neointimal formation after injury in rat arteries. In humans, high levels of osteopontin mRNA and proteins were reported in atherectomy samples from restenotic lesions [27].

In one of their first clinical studies, Kato et al. [28,29] found higher plasma osteopontin levels in patients with restenosis than in those without it, and the biomarker proved to be an independent factor for restenosis. However, in that study the investigators had not measured osteopontin before PCI. In their next prospective study, they found that preprocedure osteopontin and CRP levels are independent predictors of further cardiovascular events, but not of restenosis.

To better understand the dynamic inflammatory response after PCI, we evaluated the time course of the inflammatory markers in a subgroup of consenting patients with CAD after PCI and then at 1, 15, 30, 90, and 180 days. We confirmed the overlapping acute inflammatory response 24 h after the procedural arterial wall injury by PCI [30], and found a progressive decline and normalization of IL-6 and CRP within 30 days. In contrast, osteopontin

![Fig. 2](image-url)

Prospective relationship between baseline plasma levels of inflammatory markers [osteopontin (OPN), C-reactive protein (CRP), interleukin-6 (IL-6) (two-tailed t-test)] and rapid coronary artery disease (CAD) progression [left: progression (P), no progression (NP), and instent restenosis] [right: restenosis (R), no restenosis (NR) in patients undergoing percutaneous coronary intervention (PCI), at 6-month angiography follow-up.

![Fig. 3](image-url)

The highest baseline levels of osteopontin (OPN) were found in patients with coronary artery disease involving instent restenosis (R) and rapid plaque progression (P). These are significant versus patients without [(P = 0.003) (no restenosis (NR)/no progression (NP)]. The baseline OPN of a patient involving rapid plaque progression is significant with respect to patients without rapid plaque progression (P = 0.02).
increased acutely after PCI in patients with ACS, and continued to be higher than in those with CCS even at the 6-month follow-up visit (Fig. 4). Our data agree with those of Panda et al. [27] showing that plasma osteopontin levels in patients undergoing coronary atherectomy are elevated for at least 4 weeks. Furthermore, they suggest that osteopontin may play a role in both the vascular wall remodeling mechanism of restenosis and in the plaque remodeling involved in rapid progression of atherosclerosis. Plaque destabilization and widespread inflammatory response in ACS suggest that the extent of the systemic inflammatory status may be a crucial factor in the pathogenesis of restenosis and rapid primary plaque evolution in the coronary tree [1–9]. In our study, patients with ACS show higher and a more significant incidence of de-novo plaque progression versus those with CCS (Table 3, Fig. 4). With the reduction in restenosis rates by drug-eluting stents, there is new controversy with regard to the optimal management of incidental, nontarget lesions identified during percutaneous coronary intervention. Such lesions have been treated conservatively because of risk of restenosis but now are being considered for PCI to prevent plaque instability. The impact of incidental stenoses on future cardiac events remains poorly known. Glaser et al. [11] have shown that in a large cohort of patients undergoing PCI, the incidence of clinical plaque progression requiring additional nontarget lesion PCI is 6%, and clinical plaque progression present in the majority of cases as an acute coronary syndrome. Overall,

![Fig. 4](image)

**Time course of osteopontin (OPN), C-reactive protein (CRP), interleukin-6 (IL-6) plasma levels in 39 patients with coronary artery disease at 1, 15, 30, 60, 180 days after percutaneous coronary intervention. Inflammatory markers increased at 24 h after percutaneous coronary intervention in all patients with coronary artery disease. OPN persisted significantly elevated, up to the 3-month follow-up, in acute coronary syndrome over chronic coronary syndrome.**

![Fig. 5](image)

**Of the 77 patients with coronary artery disease, who underwent repeat angiography at 6 ± 8 months after percutaneous coronary intervention, 40% involved accelerated progression of plaques in earlier subcritical, untreated coronary lesions: 74% of patients were found to be affected by acute coronary syndrome (ACS) versus 26% who were found to be affected by chronic coronary syndrome (CCS) ($P = 0.02$).**
coronary artery disease burden during initial angiography confers a significant risk for subsequent clinical plaque progression requiring nontarget lesion PCI, but the majority of lesions are less than 50% in severity during initial angiography. The current angiographic and clinical predictors are relatively poor surrogates to predict future events in a not insignificant portion of the PCI population, further study is useful to refine the ability to identify potentially vulnerable, but clinically silent, plaques also by the support of appropriate biomarkers. The presence of multiple complex lesions as shown by angiography proved to be associated with ACS (19.0%) and death (6.0%), supporting the notion that plaque vulnerability is a ‘dynamic process’ that is not limited to the morphological characteristics. The greater the coronary artery disease burden, the higher the risk of clinical plaque progression.

Considering this complex pathological background, it would be more desirable to find sensitive biomarkers that are able to quantify both the systemic inflammation and the tissue remodeling. This would allow us to predict the risk of accelerated atherosclerosis and the recurrent clinical events, thus leading to better secondary prevention, and also providing new therapeutic approaches to the disease.

**Study limitations**

The major limitations of this study consist of the relatively small number of recruited patients, which prevented us from carrying out more exhaustive statistical analysis, including major adverse cardiovascular events and cardiovascular risk stratification. Furthermore, we were not able to determine the main source of plasma osteopontin because we did not measure osteopontin levels in the coronary sinus. However, our prospective clinical study model and current data are preliminary, and need to be extended to a broader population with CAD to confirm the predictive value of osteopontin in accelerated atherosclerosis and in cardiovascular outcomes.

**Conclusion**

After PCI, the extent of inflammation in patients with CAD seems to be associated with accelerated atherosclerosis. As a mediator of inflammatory and tissue remodeling processes involving stable/unstable plaque pathophysiology in CAD, osteopontin seems to be a sensitive biomarker and a possible predictor of accelerated atherosclerosis.

**References**


## AUTHOR QUERY FORM

**JOURNAL NAME:** MCA  
**ARTICLE NO:** 11322  
**QUERIES AND / OR REMARKS**

<table>
<thead>
<tr>
<th>QUERY NO.</th>
<th>Details Required</th>
<th>Author’s Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>Please confirm as we have retained NP and NR in Fig 2 and Fig 3 to match with caption.</td>
<td></td>
</tr>
<tr>
<td>Q2</td>
<td>Please provide citation for reference [31] in the text as per journal style, else delete from the reference list.</td>
<td></td>
</tr>
</tbody>
</table>