Elevated Plasma Active Matrix Metalloproteinase-9 Level Is Associated With Coronary Artery In-Stent Restenosis


Objective—This study aimed to determine whether the plasma levels of matrix metalloproteinase-9 (MMP-9) or tissue inhibitor of metalloproteinases-1 (TIMP-1) were altered in patients with a history of symptomatic in-stent restenosis (ISR).

Methods and Results—A group of 158 patients with a history of ISR were compared with 128 symptom-free patients. Plasma samples and a detailed risk factor history were collected. Plasma samples were analyzed for pro–MMP-9 and latent MMP-9, latent MMP-3, and TIMP-1. Several variables were associated with ISR, including index coronary disease extent and severity (number of diseased vessels and American College of Cardiology/American Heart Association lesion classification), number, diameter, and total length of stent(s) inserted, and plasma high-density lipoprotein cholesterol. Plasma active MMP-9 (odds ratio, 1.96; 95% CI, 1.43 to 2.69) showed independent risk association with ISR. Patients with multiple sites of ISR had significantly higher levels of active MMP-9 compared with patients with only a single ISR lesion or no ISR.

Conclusion—Plasma active MMP-9 levels may be a useful independent predictor of bare metal stent ISR. (Arterioscler Thromb Vasc Biol. 2006;26:0000-0000.)

Key Words: PLEASE ■ SUPPLY ■ KEY ■ WORDS ■ XXXX

Vascular connective tissue remodeling is a well-recognized ubiquitous process that occurs after percutaneous coronary interventions including intracoronary stent placement. Mechanical injury has been shown to induce collagenase and stromelysin gene expression in cultured smooth muscle cells and an increase in gelatinase activity after balloon catheter injury to the rat carotid. Overexpression of the gelatinase matrix metalloproteinase-9 (MMP-9) has been shown to enhance smooth muscle cell (SMC) migration within in vitro assays. Moreover, the levels of various MMPs appear to be associated with postinterventional vascular remodeling in human blood vessels. Such in vitro and in vivo findings suggest that increased levels of MMPs in coronary arteries undergoing percutaneous intervention may be associated with vascular remodeling and restenosis by promoting migration of vascular SMCs.

In this study, the circulating plasma levels of pro–MMP-9, latent MMP-9 and active MMP-9, latent MMP-3, and tissue inhibitor of metalloproteinases-1 (TIMP-1) were investigated in patients who had previously undergone bare metal coronary stent placement. Patients were subdivided into those who developed symptomatic, angiographically proven, in-stent restenosis (ISR) and those who were (angina) symptom-free for >1 year after their stent placement.

Materials and Methods

Subjects

Patients with coronary bare-metal stent placements were recruited retrospectively from the Dunedin Hospital Cardiology Clinical database. A group of 158 consecutive patients with a history of symptomatic, angiographically proven ISR (coronary artery disease [CAD] with ISR) were compared with a consecutive series of 128 symptom-free patients who were angina-free for >1 year after their stent placement (CAD with stent). The CAD with ISR group included patients who had undergone repeat percutaneous intervention or coronary artery bypass surgery and were then free of symptoms and cardiovascular events for >6 months. The majority (97.2%) of patients were of white ethnicity, the remainder being New Zealand Māori (2.1%) or Asian (0.7%). Coronary angiograms in all patients were analyzed by an experienced cardiologist, with the extent of CAD expressed as the number of vessel territories (left anterior descending, left circumflex, and right coronary arteries) with ≥1 stenoses of ≥50% of the vessel normal reference diameter using visual assessment of lesion severity. CAD extent was expressed as single-, double-, or triple-vessel CAD. The American College of Cardiology/American Heart Association (ACC/AHA) classification was used to evaluate the morphology of coronary lesions at the index coronary angiogram. Follow-up angiography was analyzed in the restenosis group with the definition of restenosis being diameter stenosis ≥50% of the vessel reference diameter by visual assessment at the site of the lesion treated with the stent observed in ≥1 multiple projections. The single most severe view was used to categorize the pattern of restenosis as proposed by Mehran et al for classification of in-stent restenotic lesions.

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A detailed record of each individual’s current medications, body mass index (BMI), waist-to-hip ratio (WHR), and risk factor history, including previous history of hypertension, hyperlipidemia, diabetes, other vascular diseases, and smoking history, was collected. One smoking pack year was defined as 20 cigarettes (1 pack) per day for 1 year. All subjects gave written informed consent before being recruited into this study.

Sample Analysis
Plasma samples were analyzed for lipoprotein(a) (Lp(a)) concentration (sandwich ELISA), high-sensitivity C-reactive protein (hs-CRP; Roche, Tina-quant high sensitivity [latex] assay), and lipid profiles (enzymatic-colorimetric method; Roche). Plasma Lp(a), nmol/L 27.8 (0–126.7) 28.8 (0–135.5) 0.99

Endogenous plasma MMP-3 and MMP-9 was assessed in heparin plasma samples using the Biotrak Activity Assay System (product RPN 2614 and RPN 2611; Amersham Biosciences). This system measures latent enzyme (pro-form and active forms but with a relatively low affinity with that bound to TIMPs, as reported by the manufacturer) after activation using p-aminophenylmercuric acetate. The exclusion of p-aminophenylmercuric acetate results in a measurement of the endogenous free active MMP-9 fraction. Total pro-MMP-9 and TIMP-1 levels were assessed in EDTA plasma sample using the Biotrak Activity Assay System (product RPN 2639 and RPN 2634; Amersham Biosciences). This system measures latent enzyme (pro-form and active forms but with a relatively low affinity with that bound to TIMPs, as reported by the manufacturer) after activation using p-aminophenylmercuric acetate. The exclusion of p-aminophenylmercuric acetate results in a measurement of the endogenous free active MMP-9 fraction. Total pro-MMP-9 and TIMP-1 levels were assessed in EDTA plasma samples using ELISA (product RPN 2614 and RPN 2611; Amersham Biosciences), the ratio of pro-MMP-9 (or latent) to active enzyme therefore indicating the degree of zymogen activation. The average coefficient of variance for both activity and ELISA assays was <5.5%.

Statistical Analysis
Statistical analysis was performed with StatView version 5.01 (SAS Institute). The distribution of continuous variables was assessed (kurtosis and skewness) and analyzed accordingly with either the Mann–Whitney U test or ANOVA with the Fisher protected least significant difference test.

Multiple logistic regression was used to test the interactive effects of other variables on the observed association between plasma active MMP-9 and ISR. A stepwise entry procedure was applied to identify significant or suggestive (P<0.15) confounders of either patient group or MMP level. The resulting winnowed model (WHR, BMI, plasma hs-CRP, high-density lipoprotein [HDL] cholesterol, TIMP-1, diabetes, extent of coronary disease, ACC/AHA lesion classification, total stent(s) length, number of sites stented, average stent diameter and medications) was not significantly different from an all-inclusive model.

Results are expressed as means± SD, except non-Gaussian variables, which are expressed as medians and interquartile range. Odds ratios are expressed with 95% CIs. A P value <0.05 was considered significant.

<p>| TABLE 1. Demographic Markers |</p>
<table>
<thead>
<tr>
<th>CAD With Stent</th>
<th>CAD With ISR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, % male</td>
<td>70.3</td>
<td>72.8</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.4±8.9</td>
<td>61.9±9.8</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92±0.08</td>
<td>0.94±0.08</td>
</tr>
<tr>
<td>BMI</td>
<td>28.1±4.5</td>
<td>29.5±5.2</td>
</tr>
<tr>
<td>Hypertension, &gt;140/90 mm Hg</td>
<td>40.4</td>
<td>43.4</td>
</tr>
<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>4.38±0.97</td>
<td>4.45±0.99</td>
</tr>
<tr>
<td>Plasma LDL cholesterol, mmol/L</td>
<td>2.23±0.80</td>
<td>2.36±0.86</td>
</tr>
<tr>
<td>Plasma HDL cholesterol, mmol/L</td>
<td>1.20±0.36</td>
<td>1.06±0.27</td>
</tr>
<tr>
<td>Plasma nonfasting triglycerides, mmol/L</td>
<td>2.1±1.2</td>
<td>2.2±1.3</td>
</tr>
<tr>
<td>Plasma Lp(a), nmol/L</td>
<td>27.8 (0–126.7)</td>
<td>28.8 (0–135.5)</td>
</tr>
<tr>
<td>Diabetes, % treated</td>
<td>13.3</td>
<td>19.8</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>2.2 (0.7–3.8)</td>
<td>2.7 (1.0–4.4)</td>
</tr>
<tr>
<td>Smoking pack years</td>
<td>19.9±27.4</td>
<td>20.1±25.7</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors, % treated</td>
<td>37.0</td>
<td>52.6</td>
</tr>
<tr>
<td>Ca²⁺ antagonists, % treated</td>
<td>22.0</td>
<td>36.5</td>
</tr>
<tr>
<td>β-Blockers, % treated</td>
<td>66.1</td>
<td>75.6</td>
</tr>
<tr>
<td>Nitrates, % treated</td>
<td>19.7</td>
<td>43.6</td>
</tr>
<tr>
<td>Statins, % treated</td>
<td>91.3</td>
<td>92.3</td>
</tr>
<tr>
<td>CAD extent, % single, double, triple vessel disease</td>
<td>46.0, 34.9, 19.1</td>
<td>33.8, 32.4, 33.8</td>
</tr>
<tr>
<td>ACC/AHA lesion classification, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>9.5</td>
<td>5.8</td>
</tr>
<tr>
<td>B1</td>
<td>39.7</td>
<td>35.9</td>
</tr>
<tr>
<td>B2</td>
<td>33.3</td>
<td>18.6</td>
</tr>
<tr>
<td>C</td>
<td>17.5</td>
<td>39.7</td>
</tr>
<tr>
<td>Total length of stents inserted, mm</td>
<td>21.3±11.1</td>
<td>30.2±16.9</td>
</tr>
<tr>
<td>Average stent diameter, mm</td>
<td>3.2±0.5</td>
<td>3.0±0.5</td>
</tr>
<tr>
<td>No. of sites stented</td>
<td>1.2±0.5</td>
<td>1.5±0.6</td>
</tr>
</tbody>
</table>

Several variables were differentially associated with ISR including markers of adiposity, inflammation, medications, severity of coronary disease, and the nature of the resulting intervention.

*Triple vs single and double vessel disease combined P<0.005; †log-transformed post hoc ANOVA.
Results

Demographic Confounders

Patients with ISR had no significant differences in gender, age, previous history of hyperlipidemia or hypertension, total cholesterol, low-density lipoprotein cholesterol, triglycerides, or tobacco smoking history compared with those without clinical evidence of restenosis (Table 1). In contrast, ISR patients had a significantly higher WHR, BMI, plasma hs-CRP, rate of triple vessel coronary disease, ACC/AHA lesion classification, and stent characteristics and lower plasma HDL cholesterol (Table 1). Patients with ISR were also significantly more medicated than symptom-free patients (Table 1).

Plasma Metalloproteinase and TIMP-1 Levels

Both latent MMP-3 and TIMP-1 were significantly higher in patients with triple vessel disease compared with those without clinical evidence of restenosis (Table 1). In contrast, ISR patients had a significantly higher WHR, BMI, plasma hs-CRP, rate of triple vessel coronary disease, ACC/AHA lesion classification, and stent characteristics and lower plasma HDL cholesterol (Table 1). Patients with ISR were also significantly more medicated than symptom-free patients (Table 1).

Active plasma MMP-9 and the ratio of active to latent MMP-9 were significantly greater in patients with triple vessel disease compared with those without clinical evidence of restenosis (Table 1). In contrast, ISR patients had a significantly higher WHR, BMI, plasma hs-CRP, rate of triple vessel coronary disease, ACC/AHA lesion classification, and stent characteristics and lower plasma HDL cholesterol (Table 1). Patients with ISR were also significantly more medicated than symptom-free patients (Table 1).

Active plasma MMP-9 and the ratio of active to latent MMP-9 were significantly greater in patients with triple vessel disease compared with those without clinical evidence of restenosis (Table 1). In contrast, ISR patients had a significantly higher WHR, BMI, plasma hs-CRP, rate of triple vessel coronary disease, ACC/AHA lesion classification, and stent characteristics and lower plasma HDL cholesterol (Table 1). Patients with ISR were also significantly more medicated than symptom-free patients (Table 1).

The independence of plasma active MMP-9 as a risk indicator for ISR was confirmed using multiple logistic regression (Table 3). Active MMP-9 levels >2 ng/mL, observed in ~60% of ISR patients but less than one third of symptom-free patients, resulted in an adjusted odds ratio of 6.25 (Table 3). Similarly, the ratio of active to pro–MMP-9 (or latent) MMP-9 appeared to be associated with ISR; however, this largely related to active MMP-9 levels because this association was abolished when the model also included active MMP-9.

The possible relationship between the MMP and TIMP-1 measures and the severity of ISR was examined by comparing plasma levels with Mehran classification, percentage restenosis, and the number of stented segments with ISR in each patient. The only significant association was in the subset of 31 ISR patients with multiple sites of ISR who had significantly higher levels of active MMP-9 compared with patients with either a single ISR lesion or no ISR (Table 4).

Discussion

The pathology of ISR has been shown to consist primarily of neointimal smooth muscle proliferation among abundant, proteoglycan-rich, extracellular matrix. Such injury-
induced intimal thickening has been shown to be associated with significantly enhanced MMP expression. Moreover, MMPs appear to play a key role in SMC migration after vascular injury, as indicated by various MMP inhibition studies reporting significant reductions in pathological remodeling.

In this study, we observed significantly higher levels of the active form of MMP-9 in patients who had a previous history of ISR compared with patients who had undergone similar stent placement but had not developed symptoms of ISR in at least the first postinterventional year. Moreover, the level of active MMP-9 also appeared to be associated with the number of ISR lesions that had developed in each individual. This association is independent of known demographic and clinical risk factors. Although plasma TIMP-1, a key regulator of MMP tissue activity, was significantly increased in ISR patients, the association between active MMP-9 and ISR was independent of the levels of this inhibitory protein. The increased levels of active MMP-9 and the increased ratio of active to pro–MMP-9 (or latent MMP-9) isoforms support the conclusion that there is a significant shift in the zymogen activation equilibrium in patients prone to developing ISR. The lack of association between MMP levels and hs-CRP may indicate that this is, in part, attributable to a noninflammatory mechanism. A large number of bioactive molecules are known to influence MMP activation, including other MMPs and plasmin. However, the exact mechanism for the increased MMP-9 activation observed in this study was beyond the scope of this current investigation.

The patients who developed ISR had all subsequently undergone further revascularization and were free of symptoms and cardiovascular events for ≥6 months. This is a critical inclusion criterion because we aimed, as much as possible, to exclude the effect of active symptomatic cardiac disease as a confounder in plasma biomarkers such as MMPs. Nevertheless, there were some clear differences between the ISR and non-ISR groups, including preinterventional coronary disease extent, stent length and diameter, ACC/AHA index lesion characteristics, adiposity measures, and circulating factors such as HDL cholesterol and C-reactive protein, which were adjusted for when comparing groups. Potential influences on circulating levels of MMPs, such as diurnal variation, ethnicity, age, and gender have been investigated in specifically designed studies. Although Asian and Middle Eastern ethnicity appears to be associated with MMP-9 levels, the majority of subjects in this study were white, and this is unlikely to be a confounding factor in this study. Statin and calcium channel antagonist treatment have been reported to influence MMP levels. Although we noted some slight but significant differences between use of these medications and MMP-9 levels (data not shown), we controlled for the influence of these variables in our regression analysis.

Multiple logistic regression clearly demonstrated that active plasma MMP-9 showed strong independent association with the ISR patient group. The ratio of active to total MMP-9 did not show independent association when the logistic regression model included active MMP-9, indicating that this parameter did not add any additional association beyond that of active MMP-9 alone. Active MMP-9 levels appeared most predictive at plasma concentrations >2 ng/mL, with similar odds ratios being observed in smaller numbers of patients at higher cut-off values. Although this study was limited by its retrospective nature, the observation of significantly stratified risk associated with elevated active MMP-9 levels in patients with multiple sites of ISR, after adjustment for possible confounders, suggests active MMP-9 levels warrant prospective testing as a useful prognostic marker for determining risk of ISR. This study was undertaken in a patient population before the widespread availability of drug-eluting stents (DES) in the New Zealand public health system. Although it has not been shown conclusively that the excellent initial and midterm results of DES will persist in the long term, as opposed to delaying the onset of ISR, current evidence supports their efficacy. DES reduce both angiographically determined ISR rates from one third in patients with bare metal stents to <10% and the rate of major adverse cardiac events. Nevertheless, ISR restenosis is likely to persist as a significant clinical problem in the DES era, albeit at a lower rate than observed with bare metal stents. Whether plasma active MMP-9 is a useful marker of ISR in patients treated with DES needs to be determined in future studies.

In conclusion, this study indicates that the cleaved active form of MMP-9 is an independent indicator of risk of ISR in patients treated with bare metal stents. The prognostic value of this marker needs to be further evaluated in longitudinal studies and with regard to the new generation of DES, in which ISR remains a distinct, albeit less common, clinical entity.

### Table 4. Active MMP-9 and TIMP-1 Levels vs No. of ISR Sites

<table>
<thead>
<tr>
<th></th>
<th>CAD With Stent No ISR</th>
<th>CAD With a Single ISR Lesion</th>
<th>CAD With Multiple ISR Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active MMP-9, ng/mL</td>
<td>1.50 (0.89–2.12)</td>
<td>2.13 (1.50–2.77)*</td>
<td>2.53 (1.81–3.26)†‡</td>
</tr>
<tr>
<td>Odds ratio active MMP-9, ng/mL</td>
<td>—</td>
<td>1.88 (1.35–2.62)§</td>
<td>3.09 (1.82–5.24)∥</td>
</tr>
<tr>
<td>Odds ratio active MMP-9 &gt;2 ng/mL</td>
<td>—</td>
<td>5.56 (2.61–11.84)§</td>
<td>14.80 (3.89–56.28)∥</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>225.5 (190.9–260.1)</td>
<td>240.5 (208.4–272–5)∗∗</td>
<td>224.0 (163.3–284.7)</td>
</tr>
</tbody>
</table>

Active MMP-9 levels were not only associated with the presence of ISR but also the No. of ISR lesions present. *P<0.005, †P<0.0001 vs no ISR lesions and ‡P<0.03 vs a single ISR lesion. Multiple logistic regression showed a stratified risk association for active MMP-9 (§P<0.0002; ¶P<0.0001).

CAD with no ISR* as reference population and independence modeled for confounders (WHR, BMI, hs-CRP, HDL, diabetes, extent of coronary disease, ACC/AHA lesion classification, total stent(s) length, No. of sites stented, average stent diameter and medications); the percentage of patients with active MMP-9 >2 ng/mL was 31.6%, 56.3%, 71.0% in patients with no ISR, a single ISR lesion and ≥2 ISR lesions, respectively. TIMP-1 levels were significantly increased in subjects with a single ISR lesion vs no ISR (**P<0.02) but not subjects with multiple ISR lesions.
Acknowledgments
We gratefully acknowledge the assistance of Dr Sally McCormick, Biochemistry Department, University of Otago, for measuring Lp(a) levels.

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Disclosure(s)
None.

References
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