Matrix metalloproteinase 10 is associated with disease severity and mortality in patients with peripheral arterial disease

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Objective: **Peripheral arterial disease (PAD) is associated with poor prognosis in terms of cardiovascular (CV) morbidity and mortality. Matrix metalloproteinases (MMPs) contribute to vascular remodeling by degrading extracellular matrix components and play a role in atherosclerosis as demonstrated for MMP-10 (stromelysin-2). This study analyzed MMP-10 levels in PAD patients according to disease severity and CV risk factors and evaluated the prognostic value of MMP-10 for CV events and mortality in lower limb arterial disease after a follow-up period of 2 years.**

Methods: **MMP-10 was measured by enzyme-linked immunosorbent assay in 187 PAD patients and 200 sex-matched controls.**

Results: **PAD patients presented with increased levels of MMP-10 (702 ± 326 pg/mL control vs 946 ± 473 pg/mL PAD; $P < .001$) and decreased levels of tissue inhibitor of matrix metalloproteinase 1 (312 ± 117 ng/mL control vs 235 ± 110 ng/mL PAD; $P < .001$) compared with controls. Among PAD patients, those with critical limb ischemia (n = 88) showed higher levels of MMP-10 (1086 ± 478 pg/mL vs 822 ± 436 pg/mL; $P < .001$) compared with those with intermittent claudication (n = 99), whereas the MMP-10/tissue inhibitor of matrix metalloproteinase 1 ratio remained similar. The univariate analysis showed an association between MMP-10, age ($P = .015$), hypertension ($P = .021$), and ankle-brachial index ($P = .006$) in PAD patients that remained significantly associated with PAD severity after adjustment for other CV risk factors. Patients with the highest MMP-10 tertile had an increased incidence of all-cause mortality and CV mortality ($P < .03$).**

Conclusions: **Our results suggest that MMP-10 is associated with severity and poor outcome in PAD. (J Vasc Surg 2015;61:428-35.)**

Peripheral arterial disease (PAD) is a manifestation of atherosclerotic vascular disease often associated with other comorbidities, such as diabetes, dyslipidemia, and hypertension. Its prevalence in Western societies increases with age; 20% of patients older than 65 years are diagnosed with PAD, and it is associated with a high case-fatality rate due to cardiovascular (CV) ischemic events.[1-5] PAD represents a spectrum of disease severity, encompassing both asymptomatic and symptomatic disorders. The symptomatic disorders may be manifested as either intermittent claudication (IC) or critical limb ischemia (CLI), which is the initial manifestation in roughly 1% to 2% of all patients, with a mortality rate up to 25% at 1 year.[2]

An increasing body of evidence supports the notion that inflammation plays an important role in the development and progression of PAD.[6] Moreover, PAD leads to broad adaptive changes in the arterial wall and the ischemic muscle in response to atherosclerosis and blood flow impairment, respectively. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases with proteolytic activity against a wide range of extracellular proteins[7] that contribute extensively to tissue remodeling by degrading extracellular matrix components in diverse vascular pathologic processes.[1,8-11] Recent clinical studies showed an association between PAD and circulating levels of MMP-2, MMP-9, and MMP-8 compared with healthy volunteers[9-12] but did not explore the involvement of MMP-10 (stromelysin-2) in the development and progression of PAD. An association has previously been shown between MMP-10 and different inflammatory markers, increased carotid intima-media thickness, and the presence of carotid plaques in CV-risk patients free from CV complication.[13] In vivo, MMP-10 expression has been shown in endothelial cells and macrophages within human atherosclerotic plaques,[14] and augmented MMP-10 levels have been described in patients with

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increased thrombin generation, chronic kidney disease, stroke, and diabetes,[15-18] suggesting a role for MMP-10 in vascular pathologic processes associated with impaired vascular remodeling and inflammation.

On the basis of previous studies reporting the involvement of MMP-10 in atherosclerosis and inflammatory diseases, the working hypothesis was that MMP-10 levels are increased in patients with symptomatic PAD, associated with poor outcome and poor prognosis. Therefore, levels of MMP-10 were measured in patients with PAD, with analysis of its association with CV risk factors, inflammatory markers, and subclinical atherosclerosis and its relationship with clinical outcome after a follow-up period. In addition, histologic analysis was performed to examine the local expression of MMP-10 at the femoral artery of PAD patients.

METHODS

Baseline characteristics of patients. Patients (n = 187) were prospectively enrolled and blood samples collected at the time of clinical evaluation at the outpatient service of the Department of Vascular Surgery of the Complejo Hospitalario de Navarra between 2010 and 2013. No patient was included postoperatively. Patients were classified according to the severity of the disease: IC (n = 99), with a history of IC (Fontaine class II) diagnosed by hemodynamic study (Doppler ultrasound); and CLI (n = 88), with lower limb rest pain or trophic lesions (Fontaine class III-IV) confirmed by imaging studies (arteriography, magnetic resonance angiography, or ultrasonography). Among those patients belonging to Fontaine class IV, the ones with infected lesions were excluded from the study, as were individuals with evidence of neoplastic disease, with generalized or localized inflammatory disease (moderate or severe), with severe chronic kidney disease, on hemodialysis, or receiving anti-inflammatory drugs. Ankle-brachial index (ABI) was measured at rest per standard technique in the dorsalis pedis and posterior tibial arteries of both lower limbs.[19]

A thorough medical history was recorded in all patients including details of previous myocardial infarction, arterial hypertension, cerebrovascular disease, smoking status, diabetes mellitus, body mass index, and medication. Patients actively smoking or having discontinued smoking within 2 years were considered smokers. Diabetes was defined by history of diabetes mellitus or the use of antidiabetic drugs. Hypertension was defined by any history of hypertension or the use or antihypertensive drugs.

Follow-up. Patients were followed up (mean period, 27 months [range, 11-46 months]) at the outpatient service of the Department of Vascular Surgery every 3 or 6 months, depending on the severity of PAD. At those regular checkups, patients were tested for biochemical parameters and underwent physical examination and ABI measurement. No patient was lost to follow-up. For outcome evaluation of PAD patients, CV events and death were recorded. Major adverse CV events (MACE), including amputation, ischemic coronary disease, cerebrovascular disease, and all-cause mortality as a composite end point, were defined.

A control group of 200 sex-matched subjects free from clinically manifested atherosclerotic vascular disease who attended the outpatient service of the Department of Internal Medicine at the Clínica Universidad de Navarra for a general checkup was included.[13] No follow-up of the control population was recorded.

The study was approved by the Institutional Review Boards of the corresponding hospitals, and informed consent from patients was obtained.

Laboratory analysis. Serum total cholesterol, high-density lipoprotein cholesterol, triglycerides, and glucose were measured in fasting blood samples by standard laboratory techniques. Low-density lipoprotein cholesterol was estimated by the Friedewald equation. Plasma fibrinogen activity was measured by clotting assay (Clauss), and high-sensitivity C-reactive protein (hs-CRP) was measured by immunoassay (Immulite; Diagnostic Products Corporation, Los Angeles, Calif).

MMP-10 and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) determination. MMP-10 and TIMP-1 levels were assayed in serum by enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Minneapolis, Minn) as previously described.[13,20] Interassay and intra-assay coefficients of variation for the enzyme-linked immunosorbent assays were <6%.

Protein immunolocalization in human atherosclerotic lesions. Specimens from 10 patients undergoing femoral endarterectomy (>75% stenosis) were fixed with 4% paraformaldehyde, decalcified for 24 to 72 hours at room temperature (Osteosoft, 101728; Merck Millipore, Darmstadt, Germany), and embedded in paraffin. Serial sections were analyzed by immunohistochemistry as described[21] with the following antibodies: rabbit anti-human MMP-10 (10 μg/mL; Acris Antibodies, San Diego, Calif) and mouse anti-human CD68 (0.6 μg/mL; Dako, Carpinteria, Calif). Hematoxylin and eosin staining was used to examine tissue morphologic features.

Statistical analysis. Continuous variables are presented as mean and standard deviation. Comparisons between more than two groups were done with analysis of variance or χ^2 test for continuous or categorical variables, respectively. Associations between MMP-10 levels and atherosclerotic risk factors or inflammatory markers were examined by Pearson correlation test for continuous variables and unpaired Student *t*-test for categorical variables. Receiver operating characteristic (ROC) curves were performed to analyze the ability of MMP-10 to identify patients with PAD in the whole population of subjects. Binary logistic regression analysis was performed to evaluate the contribution of MMP-10 to the risk of PAD after adjustment by CV risk factors and inflammatory markers. Odds ratios and respective 95% confidence intervals (CIs) obtained after adjustment for relevant covariates are presented. Kaplan-Meier estimates were used to compare time to event differences across MMP-10 tertiles by the log-rank test. Statistical significance was established as $P < .05$. The statistical analysis was performed with SPSS for Windows software package version 15.0 (SPSS Inc, Chicago, Ill).