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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This work was supported by the "UTE project CIMA" (University of Navarra) and Red de Investigación Cardiovascular RIC (D12/0042/0009). V.G.R. was supported by a fellowship from the Foundation for Applied Medical Research (FIMA). C.R. was supported by the Spanish Ministry of Science and Innovation (MICINN-PTQ-09-02-01941).

Author conflict of interest: none.

Presented at the Eighty-second European Atherosclerosis Society Congress, Madrid, Spain, May 31-June 3, 2014.

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The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214

http://dx.doi.org/10.1016/j.jvs.2014.09.002

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).

RESULTS

Circulating MMP-10 levels are increased in PAD patients. MMP-10 was measured in 187 PAD patients and 200 sex-matched controls. The baseline characteristics of both groups are shown in Table I. Patients were older ($P < .001$), and the prevalence of traditional CV risk factors, such as diabetes, hypertension, and smoking, was higher than in controls ($P < .001$). They were under treatment as recommended by current guidelines. The increased percentage of patients taking cholesterol-lowering drugs compared with controls (statins: 61% vs 14%; $P < .001$) may explain the decreased levels of low-density lipoprotein compared with healthy subjects, although triglycerides remained higher ($P < .05$) and high-density lipoprotein remained lower ($P < .01$). Moreover, patients presented with increased levels of fibrinogen and hs-CRP ($P < .05$), as expected in a proinflammatory/atherosclerotic state. Levels of MMP-10 were increased (Fig 1, *A*; $P < .001$), whereas its principal inhibitor, TIMP-1, was decreased (Fig 1, *B*; $P < .001$), leading to a significantly elevated MMP-10/TIMP-1 ratio in PAD subjects (Fig 1, *C*).

Binary logistic regression analysis was performed to study the contribution of CV risk factors and MMP-10 to PAD in the whole population (n = 387; Table II). The multivariate analysis showed that not only age ($P < .001$), smoking ($P < .001$), diabetes ($P < .001$), hypertension ($P = .004$), and dyslipidemia ($P < .001$) but also circulating MMP-10 levels ($P = .003$) were significantly associated with PAD.

Next, to analyze the ability of MMP-10 to identify PAD patients in the whole population (n = 387; Fig 1, *D*), ROC curve analysis was performed (area under the curve, 0.674 ± 0.027; 95% CI, 0.620-0.727; $P < .001$). The cutoff value of 714 pg/mL for MMP-10 provided 62% specificity and 64% sensitivity for predicting PAD with a relative risk of 2.86 (95% CI, 1.89-4.31).

Association of MMP-10 with CV risk factors and severity of PAD. The study was then focused on the distribution of MMP-10 in PAD patients according to disease severity. Therefore, the PAD population was stratified by patients with IC (n = 99) and those with CLI (n = 88) following the Fontaine classification. Traditional CV risk factors (age, diabetes, smoking, and inflammatory markers fibrinogen and hs-CRP) were increased in CLI patients compared with IC patients (Table III). Similarly, circulating levels of MMP-10 and TIMP-1 were also higher in CLI patients ($P < .01$; Fig 1, *A* and *B*), whereas the ratio MMP-10/TIMP-1 remained similar in both groups (Fig 1, *C*). In addition, the univariate analysis showed that MMP-10 was associated with age ($r = .178$; $P = .015$) and hypertension ($P = .021$) and inversely correlated with ABI ($r = -.239$; $P = .006$).

To study the contribution of CV risk factors and MMP-10 to the severity of PAD, a multiple logistic regression analysis was performed with MMP-10 categorized in tertiles: tertile 1, ≤678 pg/mL; tertile 2, 679-1056 pg/mL; and tertile 3, ≥1057 pg/mL. Higher MMP-10 levels were associated with PAD severity (model 1, $P < .001$), in controlling for sex and age (model 2, $P < .001$), and also after introducing other traditional risk factors and inflammatory markers as covariates (model 3, $P < .001$; Table IV).

Table I. Demographic and clinical parameters of control population and peripheral arterial disease (*PAD*) patients

	Control (n = 200)	*PAD (n = 187)*	P
Demographic and clinical data			
Sex, male, %	88	88	.92
Age, years	61 (10)	71 (11)	<.001
Smokers, %	30	80	<.001
Diabetes mellitus, %	15	46	<.001
Hypertension, %	49	72	<.001
Dyslipidemia, %	77	53	<.001
Treatment, %			
Anticoagulants	—	12	
Antiplatelets	—	76	
ACE inhibitors	7	35	<.001
ARA-II	8	23	<.001
Calcium antagonists	—	20	
Vasodilators	—	10	
Beta blockers	—	26	
Statins	14	61	<.001
Laboratory data			
LDL, mg/dL	144 (36)	100 (37)	<.001
HDL, mg/dL	49 (13)	43 (17)	<.001
Triglycerides, mg/dL	125 (65)	142 (83)	.024
Fibrinogen, mg/dL	298 (94)	503 (132)	<.001
hs-CRP, mg/L[a]	4.8 (11)	17 (29)	<.001

ACE, Angiotensin-converting enzyme; *ARA-II*, angiotensin II receptor antagonist; *HDL*, high-density lipoprotein; *hs-CRP*, high-sensitivity C-reactive protein; *LDL*, low-density lipoprotein.
Mean (standard deviation) is shown.
[a]Skew variable was logarithmically transformed.

Relationship between MMP-10 and clinical outcome in PAD. To evaluate the association of MMP-10 with clinical outcome in PAD patients, the numbers of CV events and death were recorded for a mean follow-up of 27 months (range, 11-46 months). Of 187 patients, 10% (18) were subjected to amputation, 3% presented with acute cerebrovascular events, 10% had ischemic coronary disease, and 23% died during the follow-up. Univariate analysis showed no association between MMP-10 and amputation ($P = .121$), ischemic coronary disease ($P = .338$), or acute cerebrovascular events ($P = .58$). MMP-10 levels, however, were significantly increased in considering all-cause mortality (Fig 2, *A*; $P = .021$), but there was no significant association between MMP-10 and MACE as a composite end point ($P = .067$).

PAD patients were divided according to MMP-10 tertiles and Kaplan-Meier analysis was performed to assess whether the time to amputation, CV events, or mortality was associated with MMP-10 levels. The results showed that all-cause mortality at 24 months was 39% in subjects with the highest levels of MMP-10 compared with the lowest tertiles (15%; Fig 2, *B*; $P = .028$). The amputation rate at 12 months was increased in patients at the highest

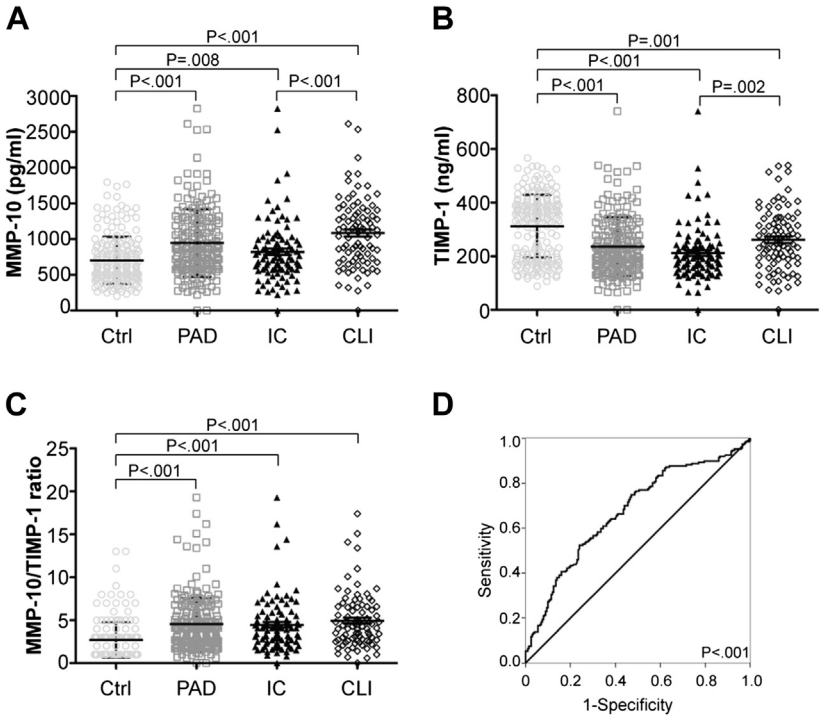

Fig 1. Circulating levels of matrix metalloproteinase 10 (*MMP-10*) are increased in patients with peripheral arterial disease (*PAD*) according to the disease severity. **A,** MMP-10 levels were measured in control subjects (*Ctrl*, n = 200) and PAD patients (n = 187). PAD patients were then divided according to disease severity into intermittent claudication (*IC*, n = 99) and critical limb ischemia (*CLI*, n = 88). **B,** Tissue inhibitor of matrix metalloproteinase 1 (*TIMP-1*) was decreased in PAD patients. **C,** MMP-10/TIMP-1 ratio was increased in patients compared with controls. **D,** Receiver operating characteristic (ROC) curve for MMP-10 plotted for various cutoff values to determine PAD.

Table II. Logistic regression analysis to estimate the odds ratio of peripheral arterial disease (PAD) in control and PAD patients

	Beta estimate (95% CI)	P
Sex, male	1.11 (0.47-2.57)	.809
Age	1.09 (1.06-1.13)	<.001
Smokers	17.11 (8.27-35.41)	<.001
Diabetes mellitus	4.06 (2.06-7.97)	<.001
Hypertension	2.42 (1.30-4.50)	.005
Dyslipidemia	0.27 (0.14-0.52)	<.001
MMP-10	1.001 (1.000-1.002)	.003

CI, Confidence interval; *MMP-10,* matrix metalloproteinase 10. Dependent variable control/PAD.

tertile compared with those in the lower ones (17% vs 7%; Fig 2, *C*; $P = .092$). Finally, the time to event for MACE was determined as a composite end point. At 24 months, 51% of the patients at the highest MMP-10 tertile suffered MACE, whereas the percentage was lower in those at the lowest tertiles (25% and 24%; $P = .015$). No differences in cerebrovascular events ($P = .715$) or ischemic coronary disease ($P = .109$) were reported among tertiles of MMP-10.

Because 57% of deaths were related to CV mortality (n = 24), survival in this subgroup was studied. As shown in Fig 2, *D*, CV mortality at 24 months was 29% and significantly increased in patients at the highest MMP-10 tertile compared with those at the lowest tertiles (3% and 11%; $P = .017$).

MMP-10 co-localizes with inflammatory cells in atherosclerotic femoral arteries. Because MMP-10 expression had not been documented in this vascular bed, arteries from PAD patients undergoing femoral endarterectomy were collected for the histologic analysis. As shown in Fig 3, MMP-10 expression was observed in the diseased artery wall of patients, being localized at the shoulder of the atherosclerotic lesion close to macrophage ($CD68^+$)–rich regions.

DISCUSSION

PAD is a major cause of acute and chronic illness. Compared with patients with coronary or cerebrovascular disease, those with PAD actually have higher rates of all-cause mortality and major CV events.[5] The current study reports increased circulating levels of MMP-10 in PAD patients according to disease severity and in association with poor outcome.

PAD remains highly underdiagnosed and undertreated, which results in higher risk for adverse outcomes.[1,22] Moreover, traditional atherosclerotic risk factors, such as

Table III. Baseline characteristics of intermittent claudication (*IC*) and critical limb ischemia (*CLI*) patients

	IC (n = 99)	*CLI (n = 88)*	P
Demographic and clinical data			
Sex, M, %	89	86	.600
Age, years	68 (10)	73 (11)	.002
Smokers, %	87	72	.010
Diabetes mellitus, %	28	67	<.001
Hypertension, %	70	75	.419
Dyslipidemia, %	60	45	.053
ABI	0.61 (0.17)	0.34 (0.11)	<.001
Treatment, %			
Anticoagulants	8	17	.062
Antiplatelets	83	69	.030
ACE inhibitors	30	40	.175
ARA-II	19	27	.190
Calcium antagonists	18	23	.441
Vasodilators	9	11	.608
Beta blockers	18	24	.340
Statins	69	53	.032
Laboratory data			
LDL, mg/dL	107 (38)	91 (34)	.005
HDL, mg/dL	51 (16)	35 (13)	<.001
Triglycerides, mg/dL	150 (93)	133 (69)	.145
Fibrinogen, mg/dL	452 (89)	558 (149)	<.001
hs-CRP, mg/L[a]	5.5 (9)	30.1 (37)	<.001

ABI, Ankle-brachial index; *ACE,* angiotensin-converting enzyme; *ARA-II,* angiotensin II receptor antagonist; *HDL,* high-density lipoprotein; *hs-CRP,* high-sensitivity C-reactive protein; *LDL,* low-density lipoprotein.
Mean (standard deviation) is shown.
[a]Skew variable was logarithmically transformed.

Table IV. Logistic regression analysis to estimate the odds ratio of critical limb ischemia (*CLI*) in peripheral arterial disease (PAD) patients

	Beta estimate (95% CI)	P
Model 1		
MMP-10 tertile 1	1.0 (reference)	
MMP-10 tertile 2	1.33 (0.63-2.81)	.448
MMP-10 tertile 3	6.12 (2.82-13.30)	<.001
Model 2		
Sex, male	0.73 (0.28-1.91)	.529
Age	1.04 (1.01-1.07)	.006
MMP-10 tertile 1	1.0 (reference)	
MMP-10 tertile 2	1.39 (0.64-2.98)	.404
MMP-10 tertile 3	5.92 (2.68-13.11)	<.001
Model 3		
Sex, male	1.07 (0.24-4.815)	.932
Age	1.01 (0.97-1.06)	.655
Smokers	0.34 (0.08-1.45)	.145
Diabetes mellitus	8.12 (2.76-23.82)	<.001
Hypertension	0.66 (0.22-1.93)	.444
HDL	0.97 (0.94-1.01)	.063
Fibrinogen	1.00 (0.99-1.00)	.707
hs-CRP[a]	30.82 (6.09-155.92)	<.001
MMP-10 tertile 1	1.0 (reference)	
MMP-10 tertile 2	3.26 (0.98-10.86)	.054
MMP-10 tertile 3	18.54 (5.08-67.67)	<.001

CI, Confidence interval; *HDL,* high-density lipoprotein; *hs-CRP,* high-sensitivity C-reactive protein; *MMP-10,* matrix metalloproteinase 10.
Dependent variable intermittent claudication (IC)/CLI.
[a]Skew variable was logarithmically transformed.

diabetes mellitus, cigarette smoking, advanced age, hyperlipidemia, and hypertension, are poor predictors of CV events in PAD.[23,24] Improved methods of risk classification to enhance proper PAD diagnosis and treatment are needed. MMPs have attracted increasing interest in the CV field because they are involved in atherosclerotic plaque formation, abdominal aortic aneurysm development, and tissue remodeling in CLI.[25,26] However, there is limited knowledge about the pathophysiologic information they can provide as potential biomarkers in PAD. MMP-2, MMP-9, and MMP-8 have been shown to be increased in PAD patients,[9-12] but their utility as diagnostic and prognostic tools has not been properly established. Moreover, the diverse functions and systemic sources of MMPs argue for a careful examination of each family member in CV pathologic processes. An association has been described between circulating levels of MMP-10 and different inflammatory markers, increased carotid intima-media thickness, and the presence of carotid plaques in subjects with subclinical atherosclerosis.[13] Besides, patients with disseminated intravascular coagulation, chronic kidney disease, stroke, or diabetes present increased circulating levels of MMP-10,[15-18] suggesting a role in vascular pathologic processes associated with vascular remodeling and inflammation. Likewise, this study reports increased levels of MMP-10 independent of other risk factors in PAD patients, although other clinical parameters such as smoking, hypercholesterolemia, and diabetes were more strongly associated with PAD. Accordingly, ROC curve analysis showed only a moderate sensitivity and specificity for MMP-10 in the identification of PAD patients. Interestingly, MMP-10 has been suggested to be a marker of disease severity and poor outcome in atherosclerotic and inflammatory pathologic processes.[13,15,17,20] Therefore, we focused the analysis of MMP-10 in PAD patients, showing elevated circulating levels of MMP-10 in association with traditional CV risk factors but not with the atherosclerosis-associated inflammatory markers CRP or fibrinogen. However, an inverse correlation was found between MMP-10 and ABI, the most commonly used diagnostic test in PAD and also a marker of atherosclerotic burden with prognostic implications for CV events.[2] The association of MMP-10 with ABI supports previous results showing its association with subclinical and clinical atherosclerosis[13,20] and its possible use as an indicator of severity and risk for CV morbidity and mortality in PAD. Whether the combined measurement of MMP-10 and ABI could improve the diagnosis and posterior treatment of those asymptomatic patients in whom ABI is not yet altered or in patients at high risk of presenting with an event should be further studied in long-term studies.

PAD may be manifested as IC or CLI, the latter conferring higher risk of death and CV events (20% to 25% of patients die at 1 year, and 25% to 30% undergo

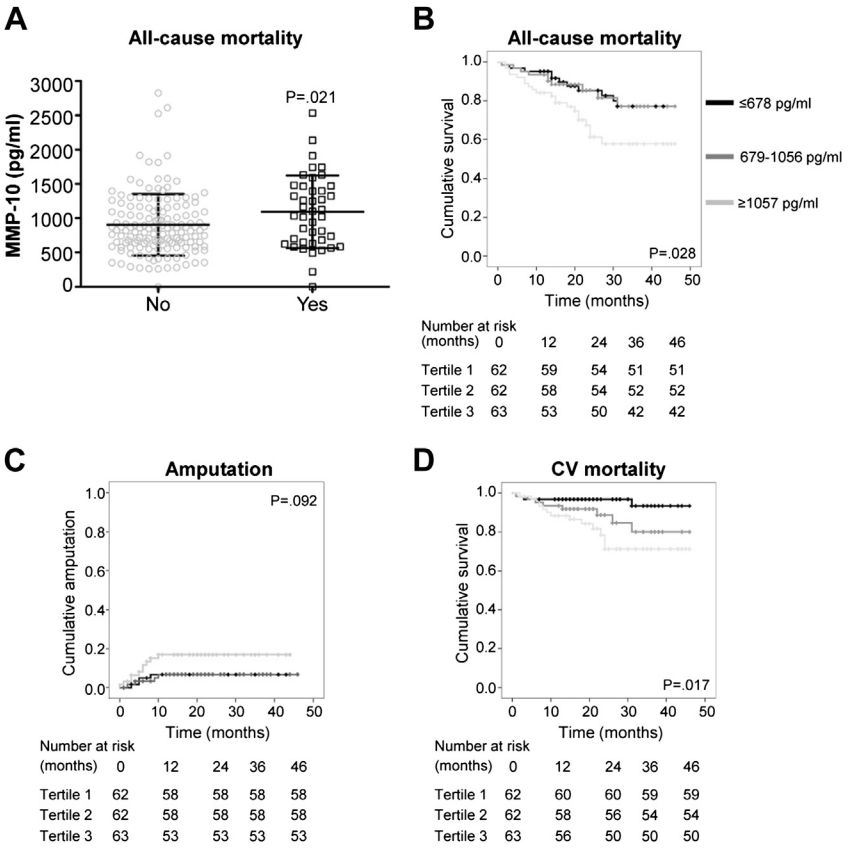

Fig 2. Matrix metalloproteinase 10 (*MMP-10*) is associated with clinical outcome in peripheral arterial disease (PAD). **A,** Circulating MMP-10 levels are increased in PAD patients who died. **B** and **C,** Kaplan-Meier estimates for cumulative death **(B)** and amputation **(C)** in PAD patients in relation to MMP-10 tertiles (**C,** log-rank test, $P < .05$). **D,** Kaplan-Meier estimates for cumulative cardiovascular (*CV*) mortality (log-rank test, $P < .05$).

major amputation).[27] As a more severe or unstable state, CLI may be associated with increased matrix turnover, inflammation, and endothelial stress, which can lead to increased proteolytic activity. In agreement with a previous study showing increased levels of MMP-9 in CLI patients,[11] the current study reports significantly higher levels of MMP-10 and TIMP-1 in these patients. The observed rise in circulating TIMP-1 could counterbalance the increased proteolytic activity of vascular patients or reflect the enhanced fibrosis shown by CLI subjects.[11]

The current goals of symptomatic PAD management are to eliminate or to reduce ischemic symptoms, to avoid major amputation in the most severe cases, and to prevent CV events (myocardial infarction, stroke, or death) that result from widespread atherosclerosis.[23] Different biochemical biomarkers, such as hs-CRP, fibrinogen, and amino-terminal pro-B-type natriuretic peptide,[23,28-30] have been evaluated as independent predictors of CV events and mortality in PAD. Even though the measurement of these markers could be useful to identify vulnerable patients, in some studies the addition of these parameters to established risk factors improves their identification only slightly.[28] Moreover, the contribution of those markers to predict adverse events varies by the follow-up period (short-term vs longer term),[29] age, or diabetic status.[23] The evaluation of MMP-10 as an early prognostic marker of mortality and CV events in PAD showed an association with all-cause mortality and CV mortality when it was examined separately. These results suggest a contribution of MMP-10 to the overall prediction of CV death in PAD, although larger studies, in terms of number of patients and follow-up period, should be designed to confirm these data.

PAD is a manifestation of systemic atherosclerosis, and MMPs have been involved in all the steps of plaque development. As previously reported in human carotid atherosclerotic plaques,[14] the morphologic analysis of femoral arteries from PAD patients showed MMP-10 expression in rupture-prone regions, rich in macrophages, within atherosclerotic plaques. Our current and previous results indicate that systemic and local MMP-10 activity may contribute to plaque rupture and its associated complications.

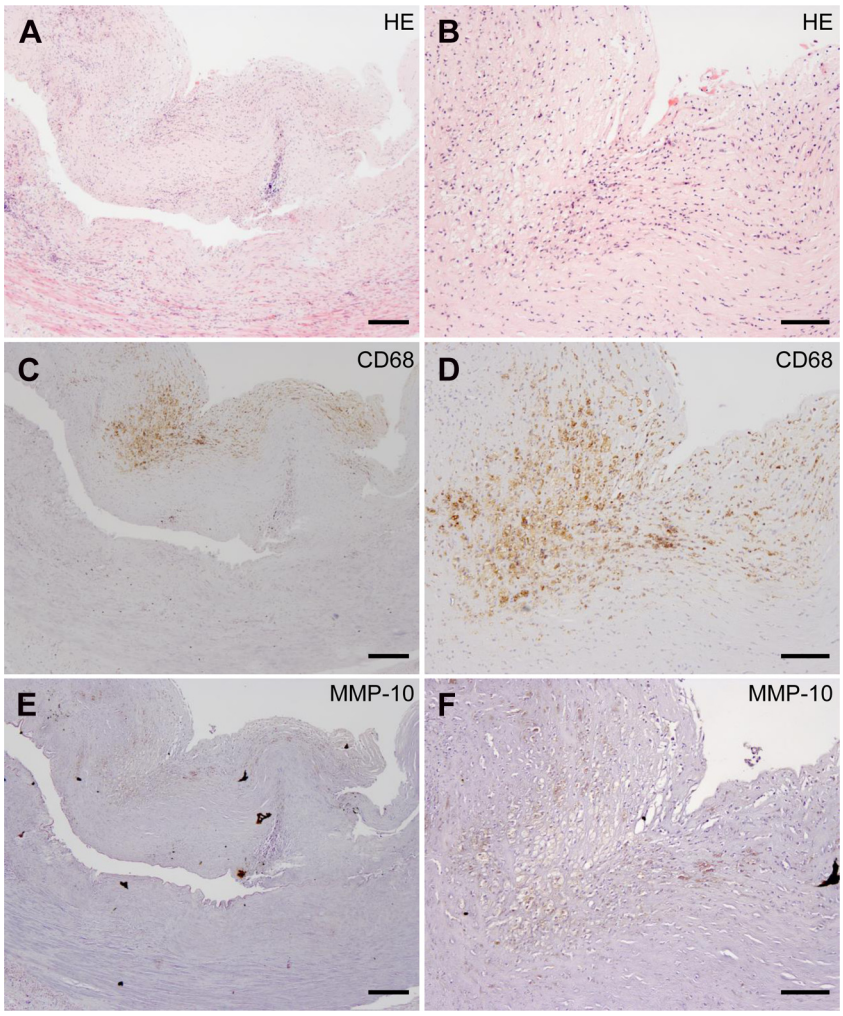

Fig 3. Morphologic analysis showing matrix metalloproteinase 10 (*MMP-10*) expression in atherosclerotic femoral arteries. **A,** Hematoxylin-eosin (*HE*) staining of femoral artery section. **B,** Higher magnification detail of HE-stained sections. **C** and **D,** Immunohistochemistry for CD68 macrophage marker and a higher magnification detail **(D)**. **E** and **F,** Immunohistochemistry for MMP-10 and a higher magnification detail **(F)**. *Scale bar* in **A, C,** and **E** denotes 250 μm. *Scale bar* in **B, D,** and **F** denotes 10 μm.

Limitations of the study. Patients and controls were not age matched. However, this variable was included in the multivariate analysis, and MMP-10 remained associated with PAD independently of other risk factors. The mean follow-up period of 2.3 years is useful to estimate early rather than long-term amputation and mortality in PAD patients, and the role of MMP-10 as a prognosis marker of CV events should be evaluated in longer term studies. Some patients classified as non–CV mortality died of unknown causes; thus, it could not be determined whether in some cases they were also of CV nature. Association analysis between baseline MMP-10 measurement and outcome in PAD has not taken into account potential variations in MMP-10 during the follow-up period. The usefulness of regular MMP-10 measurements as more accurate estimators for the subsequent risk of PAD evolution, CV events, and mortality should be assessed. The influence of medical treatment, especially of statins, on MMP-10 levels should be studied in larger cohorts of patients.

CONCLUSIONS

This study shows for the first time that MMP-10 is increased in PAD, in association with poor outcome, thus representing a new marker of severity and prognosis in PAD patients. Large prospective observational and clinical trials targeting MMP-10 will provide new insights into the pathogenesis and treatment of PAD.

We want to thank Dr O. Beloqui (Clínica Universidad de Navarra, Pamplona, Spain) for kindly providing the control samples, Lara Montori (Laboratory of Atherothrombosis,

CIMA) for her technical assistance, and Begoña Bermejo (Complejo Hospitalario de Navarra) for her help in the statistical analysis.

AUTHOR CONTRIBUTIONS

Conception and design: EM, LF, CR, JP
Analysis and interpretation: EM, JO, CR, JP
Data collection: EM, VG
Writing the article: EM, CR, JP
Critical revision of the article: EM, JO, JR, LF, CR, JP
Final approval of the article: EM, VG, JO, JR, LF, CR, JP
Statistical analysis: EM, JO, CR
Obtained funding: JP
Overall responsibility: JP

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Submitted Jul 17, 2014; accepted Sep 4, 2014.