Is Thrombogenesis in Atrial Fibrillation Related to Matrix Metalloproteinase-1 and Its Inhibitor, TIMP-1?

Francisco Marín, MD; Vanessa Roldán, MD; Vicente Climent, MD; Amaya García, MD; Pascual Marco, MD; Gregory Y.H. Lip, MD

Background and Purpose—Decreased matrix metalloproteinase-1 (MMP-1) and increased levels of its inhibitor, tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), reflect impaired matrix degradation with an increase in fibrosis. A prothrombotic state has been described in atrial fibrillation (AF), increasing the risk of stroke and thromboembolism. Because structural abnormalities and remodeling of atria have been observed in AF, we hypothesized that the prothrombotic state in AF may be related to abnormal indexes of matrix degradation.

Methods—We studied 48 consecutive patients (30 men; age, 70.5±9.0 years) with chronic nonrheumatic AF who were not on anticoagulation. Plasma levels of MMP-1, TIMP-1, and prothrombin fragment 1+2 (F1+2, an index of thrombogenesis) were measured by enzyme-linked immunosorbent assay. M-mode, 2-dimensional, and Doppler echocardiographic studies were performed in all patients. Research indexes were compared with data from 32 control subjects in sinus rhythm who were of similar age and sex.

Results—Patients with AF had lower levels of MMP-1 (P=0.011) but increased levels of TIMP-1 (P=0.033) and F1+2 (P<0.001) and a higher ratio of TIMP-1 to MMP-1 (P=0.009) compared with control subjects. After adjustment for sex, age, hypertension, and diabetes, TIMP-1 levels and the ratio of TIMP-1 to MMP-1 correlated with F1+2 levels (r=0.24, P=0.038; and r=0.26, P=0.023, respectively). In multivariate analysis, there was no independent relationship between MMP-1, TIMP-1, or ratio of TIMP-1 to MMP-1 and the presence of AF.

Conclusions—Patients with AF have evidence of impaired matrix degradation, but this was not independently associated with the presence of AF on multivariate analysis. However, an independent relationship was found between the MMP/TIMP system and prothrombotic state (assessed by F1+2 levels). (Stroke. 2003;34:1181-1186.)

Key Words: atrial fibrillation • metalloproteinases • tissue inhibitor of metalloproteinases

The extracellular matrix is a dynamic structure, with continuous changes in the amount and proportions of its structural proteins that include different types of collagen, elastin, proteoglycans, and glycoproteins. The extracellular matrix also participates in the control of numerous cellular functions. Different enzymes such as the matrix metalloproteinase (MMP) family help with the degradation of extracellular components.

MMPs and their inhibitors, mainly tissue inhibitor of metalloproteinase-1 (TIMP-1), have been related to cardiovascular disease. Indeed, the MMP/TIMP system seems to be crucial to the extracellular matrix degradation seen in cardiovascular disease. For example, an increase in MMP activity has been noted in the shoulders of atherosclerotic plaques prone to rupture or during remodeling after acute myocardial infarction. Laviades et al demonstrated decreased serum concentration of MMP-1 (the most important enzyme in the extracellular degradation of collagen) with raised levels of TIMP-1 in patients with essential hypertension. Recently, plasma TIMP-1, the molecule that inhibits collagen degradation, has been proposed as a noninvasive marker of interstitial fibrosis.

Structural abnormalities have been described in the atria from patients with atrial fibrillation (AF), which are most severe in patients with chronic permanent AF. However, it is unclear whether these changes are caused by the arrhythmia or by underlying heart disease, although experimental models suggest that AF itself may lead to these structural alterations and importantly, histological studies in atrial biopsies of patients with paroxysmal AF and lone AF refractory to standard treatment have demonstrated structural changes (eg, lymphomononuclear infiltrates and nonspecific patchy fibrosis) that are not seen in biventricular biopsies. Recently, increased expression of ADAMs (N Disintegrin And Metalloproteinases), a new family of proteases that regulate cell-matrix interactions, has been reported in AF, suggesting that...
their increased activity could contribute to atrial remodeling.12 These atrial structural abnormalities may contribute to thrombogenesis in AF and, together with the prothrombotic or hypercoagulable state seen in this arrhythmia,13 may lead to the high risk of stroke and thromboembolism in this condition. Indeed, in the Third Stroke Prevention in Atrial Fibrillation (SPAF III) study, elevated prothrombin fragment 1+2 (F1+2) levels, an index of thrombogenesis, were associated with a clinical risk factor for stroke in AF.14 On the other hand, 1 of the most important activators of MMPs is plasmin15; thus, the prothrombotic state found in AF could be intimately related to matrix remodeling.

Because structural abnormalities and remodeling of atria have been observed in AF, we hypothesized the following: (1) that abnormalities in the MMP/TIMP system (specifically, MMP-1 and TIMP-1) may be implicated in these changes; (2) that abnormalities in the MMP/TIMP system could be independently associated with the arrhythmia and not underlying cardiovascular conditions such as hypertension, diabetes, or heart failure; and (3) that the prothrombotic state demonstrated in AF (as assessed by measurement of F1+2 levels) may be related to abnormal indexes of matrix degradation. To test these hypotheses, we studied 48 consecutive patients with chronic nonrheumatic AF who were not on anticoagulant therapy.

**Patients and Methods**

We studied 48 consecutive patients (30 men; age, 71±9 years) with chronic nonrheumatic AF (duration >4 weeks, confirmed electrographically) who were referred to our anticoagulation clinic for the initiation of oral anticoagulation. Clinical risk factors for thromboembolic events—age, sex, hypertension, diabetes, heart failure, ischemic heart disease, and previous arterial embolism—were noted. Exclusion criteria were as follows: patients with acute AF (or AF lasting <4 weeks); hemodynamic impairment within the previous 6 weeks; valvular heart disease; recent (<3 months) venous thromboembolic or systemic arterial embolism, myocardial infarction, stroke or acute coronary syndrome, and surgery; and acute infection or inflammatory disease, malignancy, and renal/liver impairment.

Research indexes were compared with findings from 32 control subjects in the whole group, MMP-1 levels were significantly lower (P=0.011) but increased levels of TIMP-1 (P=0.033) and F1+2 (P=0.001), as well as a higher ratio of TIMP-1 to MMP-1 (P=0.009), compared with control subjects (Figure 1). Plasma fibrinogen levels were not significantly different between cases and controls (P=0.772).

**Echocardiography**

Transthoracic M-mode, 2-dimensional, and Doppler echocardiography (Hewlett Packard SONOS 2500) was performed in all AF patients. Echocardiographic measurements were performed in the long parasternal and 4-chamber apical axes according to guidelines from the American Society of Echocardiography.19 Ejection fraction was calculated by area-length method. Left ventricular mass was calculated by the Devereux and Reichek method20 and indexed for body surface area. Left ventricular systolic function was assessed by ejection fraction and shortening fraction. Color and continuous Doppler was used to assess the severity of valvular disease. All echocardiographic recordings were performed by the same investigator, and the coefficient of variation for our laboratory was <5%.

**Power Calculation**

The simple size calculation was based on previous cross-sectional studies of the MMP/TIMP system in cardiovascular disease.7,8 We hypothesized lower levels of MMP-1 and increased levels of TIMP-1 in AF patients by one quarter of an SD (after logarithmic transformation) compared with control subjects. To achieve this, a minimum of 20 patients and 20 control subjects was required for a 2-side value of P<0.05 with a power (1–β) of 80%. Nevertheless, to improve power and because we also measured other parameters and tested the relation to echocardiographic parameters, our aim was to recruit a minimum of 40 patients.

**Statistical Analysis**

Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test. When data (ie, MMP-1, TIMP-1, ratio of TIMP-1 to MMP-1, and F1+2 values) were not normally distributed, they were log transformed before statistical analysis. However, these data are presented in the nonlogarithmic format as median (interquartile range [IQR]). Comparisons between the 2 groups were performed with the unpaired t test (if relevant after log transformation). Categorical data were compared by use of the χ2 test, and Fisher’s exact test was performed if relevant. Correlations between the measured laboratory indexes (if relevant after log transformation) and clinical and demographic data were performed with the Pearson correlation coefficient and partial correlation coefficients, controlling for confounding variables. A stepwise multiple regression analysis was undertaken with the research indexes (MMP-1, TIMP-1, etc) as dependent variables and clinical factors (age, sex, presence of arrhythmia, hypertension, diabetes, heart failure, ischemic heart disease, and previous arterial embolism) and echocardiographic parameters (left ventricular end-diastolic and end-systolic diameters, left atrial diameter, ejection fraction, shortening fraction, and left ventricular mass index) as independent variables.

Blood Samples and Laboratory Assays

Venepuncture was performed in the morning on patients who had been fasting for >12 hours and had rested for at least 20 minutes. Blood samples were drawn atraumatically and without stasis into syringes preloaded with trisodium citrate (0.101 mol/L). Platelet-poor plasma fractions were obtained by centrifugation at 4°C for 20 minutes at 2200g within 5 minutes after blood collection. Aliquots were stored at −30°C to allow batch analysis.

MMP-1 levels were assayed by enzyme-linked immunosorbent assay (ELISA) with a commercial kit (Biorad, Amersham Pharma- cia Biotech) with a minimum sensitivity of 1.7 ng/mL. TIMP-1 levels were assayed by ELISA with reagents (R&D Systems) with a minimum sensitivity of 20 ng/mL. The ratio between TIMP-1 and MMP-1 levels was also calculated. F1+2 levels were also assayed by ELISA (F1+2 Enzygnost, Dade Behring). Fibrinogen levels were measured by a modified Clauss method. Interassay and intra-assay coefficients of variation of all assays were ≤5%, and all laboratory work was undertaken by researchers who were blinded to the patient’s clinical details.
TABLE 1. Clinical Features in Patients With AF Compared With Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>AF (n=48)</th>
<th>Control Subjects (n=32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD), y</td>
<td>71±9</td>
<td>70±6</td>
<td>0.630</td>
</tr>
<tr>
<td>Age &gt;65 y, n (%)</td>
<td>36 (75)</td>
<td>26 (81)</td>
<td>0.512</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>30 (63)</td>
<td>15 (47)</td>
<td>0.168</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>146±23</td>
<td>142±16</td>
<td>0.264</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>81±16</td>
<td>78±10</td>
<td>0.186</td>
</tr>
<tr>
<td>Medical history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>30 (63)</td>
<td>13 (41)</td>
<td>0.055</td>
</tr>
<tr>
<td>Diabetes</td>
<td>11 (23)</td>
<td>5 (16)</td>
<td>0.424</td>
</tr>
<tr>
<td>Previous thromboembolism</td>
<td>3 (6)</td>
<td>1 (3)</td>
<td>0.530</td>
</tr>
<tr>
<td>Heart failure</td>
<td>15 (31)</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>6 (13)</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>Drugs, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>18 (38)</td>
<td>3 (9)</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td>17 (35)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>β-Blocker</td>
<td>4 (8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>12 (25)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Amiodarone</td>
<td>10 (28)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SD. Comparisons by χ² test or unpaired t test.

P=0.005) and in patients with impaired New York Heart Association functional class (1.80 ng/mL [IQR, 1.70 to 2.30 ng/mL] versus 2.00 ng/mL [1.80 to 2.75 ng/mL]; P=0.044). TIMP-1 values and the ratio of TIMP-1 to MMP-1 were higher in diabetic patients (420 ng/mL [IQR, 165 to 720 ng/mL] versus 190 ng/mL [IQR, 146 to 318 ng/mL]; P=0.034; and 127.5 [IQR, 85.1 to 341.6] versus 85.1 [IQR, 59.9 to 159.6]; P=0.011, respectively). Hypertensive and ischemic heart disease patients showed lower values of MMP-1 of borderline difference (P=0.069 and P=0.089, respectively). However, there were no significant correlations between blood pressure values and values of the research indexes. In addition, the latter were not significantly affected by aspirin use (data not shown).

Correlation Coefficients
In the whole group, there was no significant correlation between log MMP-1 and log TIMP-1 values (r=−0.049, P=0.666). There was a positive correlation between log TIMP-1 and log F1+2 (r=0.27, P=0.018) (Figure 2), whereas log TIMP-1 and fibrinogen levels correlated with age (r=0.25, P=0.028; and r=0.36, P=0.002, respectively). The log ratio of TIMP-1 to MMP-1 was also significantly correlated to log F1+2 levels (r=0.29, P=0.010) (Figure 3). After adjustment for sex, age, hypertension, and diabetes, the correlation of log TIMP-1 and the log ratio of TIMP-1 to MMP-1 with log F1+2 levels remained statistically significant (partial r=0.24, P=0.038; and r=0.26, P=0.023, respectively). There were no significant correlations between research indexes and the duration of arrhythmia.

After adjustment for sex, age, hypertension, and diabetes, there were significant correlations between log MMP-1 levels and left ventricular end-diastolic diameter (partial r=−0.32, P=0.033). After adjustment for the same confounding variables, the log ratio of TIMP-1 to MMP-1 was correlated with left ventricular mass index (partial r=0.38, P=0.010). No statistically significant correlations were found between left atrial diameter (on M mode), systolic function parameters, and our research indexes (data not shown).

Multivariate Analysis
Using stepwise multiple regression analyses, we found no independent relationships between MMP-1, TIMP-1, or the ratio of TIMP-1 to MMP-1 and the presence of AF. Log MMP-1 levels were independently associated with ischemic heart disease (adjusted r²=0.182, P<0.001), end-diastolic left ventricular diameter (adjusted r²=0.182, P=0.001), and hypertension (adjusted r²=0.081, P=0.015), whereas only age was independently associated with log TIMP-1 levels (r²=0.259, P=0.010). The log ratio of TIMP-1 to MMP-1 was associated with left ventricular mass index (adjusted r²=0.143, P=0.007) and age (adjusted r²=0.100, P=0.019). Log F1+2 levels were independently associated with the presence of AF (adjusted r²=0.253, P<0.001) and impaired functional class (adjusted r²=0.044, P=0.033). Age was the only variable that was a significant predictor of plasma fibrinogen levels (r²=0.200, P=0.002).

Discussion
In the present study, patients with AF have evidence of impaired matrix degradation, but this finding was not independently associated with the presence of AF on multivariate analysis. In addition, after adjustment for confounding variables, there were also statistical correlations between the MMP/TIMP system and echocardiographic indexes of left ventricular hypertrophy (left ventricular mass index) and left ventricular remodeling (end-diastolic ventricular diameter) but no relationship to left atrial size or systolic function parameters. Interestingly, an independent relationship was found between the MMP/TIMP system and prothrombotic state as assessed by F1+2 levels in AF.

An increase in extracellular matrix produces interstitial fibrosis by virtue of raised amounts of collagen type I and III. Interstitial fibrosis increases myocardial stiffness, producing diastolic dysfunction and eventually systolic impairment. Importantly, decreased concentrations of MMP-1 and raised levels of TIMP-1 have been noted in hypertensive...
patients, which appear to be associated with depressed extracellular degradation of collagen type I, mainly in patients with left ventricular hypertrophy.\(^7\) We found a significant and independent correlation between the ratio of TIMP-1 to MMP-1 and left ventricular mass index. High concentrations of the carboxy-terminal propeptide of procollagen type I, a marker of extracellular collagen type I synthesis, have been demonstrated to significantly correlate with myocardial fibrosis.\(^23\) In a large cohort of untreated hypertensive patients,\(^8\) TIMP-1 levels significantly correlated with parameters of diastolic function, and concentrations $>500$ ng/mL had excellent values of specificity and positive predictive value for diastolic dysfunction.

After acute myocardial infarction, there is also a negative correlation between MMP-1 and TIMP-1 values and systolic function.\(^24\) Despite the relationship between the MMP/TIMP system and increased fibrosis, other authors have not found statistically significant associations with echocardiographic parameters.\(^25\) However, this study was performed in patients in stable sinus rhythm and with different exclusion criteria such as systolic dysfunction or diabetes.

The present study suggests that the MMP/TIMP system was not independently associated with the presence of AF. In an experimental model of AF,\(^10\) no modifications in the connective tissue were found, despite abnormalities in atrial myocytes. Furthermore, Schotten et al\(^26\) also found no differences in the amount of extracellular matrix during histological studies of right atrial appendages from patients in sinus rhythm and AF, whether with associated mitral stenosis nor coronary heart disease. In human necropsies,\(^27\) there appears to be evidence of increased interstitial fibrosis in atrial tissue, suggesting that those patients with higher blood pressure, other metabolic disturbances, or uncontrolled heart failure are...
more likely to develop complications such as AF. In keeping with the present study, other experimental data suggest that underlying diseases promote AF by causing atrial interstitial fibrosis. 

In the present study, the MMP/TIMP system was associated with age, diabetes, and heart failure, which are well recognized as risk factors for stroke and thromboembolic events in AF. 

We also demonstrated an independent relationship between the MMP/TIMP system and the hypercoagulable state in AF, as assessed by F1+2 levels. In the SPAF III study, F1+2 levels were also associated with clinical risk factors for thromboembolism. 

The present study suggests that the MMP/TIMP system could be a marker of associated comorbidities that increase the risk of stroke and thromboembolism in AF. This would be in keeping with several known interactions between the coagulation cascade and MMPs. 

For example, 1 of the most important activators of MMPs is plasmin. Furthermore, it has been proposed that prothrombotic state in AF could be related to vascular endothelial growth factor, a marker of angiogenesis. 

Certainly, experimental data suggest that vascular endothelial growth factor-induced thrombin generation can increase TIMP-1 secretion. However, Páramo et al were unable to find statistically significant associations between plasma levels of fibrinolytic parameters and the MMP/TIMP system, but that study was performed in stable angina patients, and they could not adequately assess MMP-1 values because all were below the lower limit of detection. As expected, antiplatelet therapy did not modify the prothrombotic markers or the MMP/TIMP system.

This study is limited by its cross-sectional design, but we have investigated for the first time the relationship between MMP/TIMP and AF and the association with the prothrombotic state. Furthermore, a cross-sectional design allows us only to explore associations, and no causality is implied; only a prospective cohort study with large numbers of subjects with AF can confirm the natural history of the indexes measured in the short, medium, and long term in relation to interventions (cardioversion, introducing antithrombotic therapy, etc), as well as morbidity and mortality. Unfortunately, transesophageal echocardiography, which could provide more structural and functional information regarding the atria, was not undertaken. The correlations are also modest, and it is likely that other influencing factors such as endothelial damage/dysfunction and stasis would play a part; larger studies are required to explore this hypothesis. Furthermore, changes with cardioversion to sinus rhythm and the prognostic value of these indexes need to be studied. Finally, our patients were diagnosed fairly recently with AF, and it is possible that with more permanent AF (and more structural abnormalities), even more marked abnormalities of MMP/TIMP would be apparent with an even greater relation to thrombogenesis.

In summary, patients with AF have evidence of impaired matrix degradation, but this was not independently associated with the presence of AF on multivariate analysis. Independent statistical associations were noted with echocardiographic parameters and abnormal thrombogenesis, suggesting a relation of matrix degradation to the structural abnormalities and prothrombotic state seen in AF.

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References


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