Background and Purpose—After acute stroke, an increased level of C-reactive protein (CRP) measured at discharge predicts unfavorable outcome. We sought to investigate whether CRP measured before tissue plasminogen activator (tPA) treatments may add prognostic information to guide stroke thrombolysis.

Methods—Our target was 151 consecutive patients with an ischemic stroke involving the middle cerebral artery territory who received tPA within 3 hours of symptom onset. High-sensitivity CRP was measured before tPA administration, and CRP gene polymorphisms were determined (G1059C and C1444T). Functional outcome was evaluated by 3-month modified Rankin Scale (mRS).

Results—A total of 143 tPA-treated patients were valid for analyses after exclusion of those with inflammatory diseases and those probably infected (CRP \(>6\) mg/dL). Patients with history of previous stroke, hypertension, or atrial fibrillation had higher levels of CRP \((P<0.05)\). CRP was higher in patients who died after thrombolysis \((n=19)\) than in survivors \((0.85 \text{ versus } 0.53 \text{ mg/dL}; P=0.002)\). Among the 94 patients with proximal middle cerebral artery occlusions, CRP level was 0.53 for 81 survivors versus 0.81 mg/dL for 13 who died \((P=0.001)\). A correlation between CRP and mRS was found \((r=0.36, P=0.02)\), although CRP polymorphisms were not related to neurological outcome. In a logistic regression model, CRP \((\text{odds ratio}=8.51; 95\% \text{ CI}, 2.16 \text{ to } 33.5; P=0.002)\) and age \((\text{odds ratio}=6.25; 95\% \text{ CI}, 1.44 \text{ to } 27.19; P=0.015)\) were the only baseline mortality predictors.

Conclusions—Admission CRP predicts mortality among tPA-treated stroke patients. Very early recanalization does not ameliorate the negative prognostic impact of elevated CRP. (Stroke. 2006;37:1205-1210.)

Key Words: C-reactive protein • inflammation • polymorphism • stroke • tissue plasminogen activator

Inflammation plays a pivotal role in vascular injury. Accordingly, high-sensitivity C-reactive protein (CRP) has emerged as a strong independent risk factor for future cardiovascular events. Long considered an innocent bystander in the atherosclerotic process, new evidence suggests that CRP might have direct proinflammatory effects. These detrimental effects might explain its usefulness to predict cardiovascular mortality or poor outcome in patients with acute coronary disease.

In the present study we sought to investigate whether CRP measurements before administration of tissue plasminogen activator (tPA) may add prognostic information to guide stroke thrombolysis. Because CRP polymorphisms have been associated with increased levels of CRP in humans, we studied the effect of 2 common CRP genetic variants, G1059C and C1444T, on the response to tPA among stroke patients.

Subjects and Methods

Study Population

Patients with an acute stroke admitted to the emergency department of a university hospital were prospectively studied. For the purposes of this study we selected 151 consecutive patients who had a documented middle cerebral artery (MCA) occlusion on transcranial Doppler ultrasonography (TCD) and received tPA in a standard 0.9-mg/kg dose (10% bolus, 90% continuous infusion during 1 hour).
within 3 hours of symptom onset following National Institute of Neurological Disorders and Stroke (NINDS) recommendations.

Clinical Protocol
A detailed history of vascular risk factors was obtained from each patient. To identify the potential mechanism of cerebral infarction, a set of diagnostic tests was performed, and previously defined etiologic subgroups were determined. Patients with a clinically known inflammatory or malignant disease were excluded from the final analysis.

Clinical examination was performed on admission and at 12, 24, and 48 hours from symptom onset. Stroke severity as well as improvement, stability, or neurological worsening was assessed with the use of the National Institutes of Health Stroke Scale (NIHSS). At the follow-up visit (3 months), functional outcome was evaluated by use of the modified Rankin Scale (mRS).

On admission, all patients underwent a CT within the first 3 hours of stroke onset. No patient with a hypodensity involving >33% of the MCA territory received tPA in this study. CT was repeated after 24 to 48 hours (or earlier when rapid neurological deterioration occurred) to evaluate the presence of hemorrhagic transformations, according to previously published criteria.

TCD measurements were performed by an experienced neurologist with the use of a Multi-Dop X4 TCD (DWL Elektronische Systeme GmbH) device, with a hand-held transducer in a range-gated, pulsed-wave mode at a frequency of 2 MHz. TCD examinations were performed before the beginning of the treatment and again by the end of tPA infusion and serially for the first 24 hours. Proximal or distal MCA occlusions and follow-up recanalization degrees were defined as previously described.

High-Sensitivity CRP Assays
Peripheral blood samples were drawn from each patient at study entry (before tPA administration). Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at −80°C until analysis. CRP levels were obtained by nephelometry with the use of a Behring Nephelometer Analyzer and expressed in milligrams per deciliter. Laboratory data were performed blinded to clinical details and patient evolution. Patients with very high CRP level (>6 mg/dL) in whom an infection before stroke could not be ruled out were also excluded from further analysis.

CRP Gene Polymorphisms
To detect G1059C and C1444T polymorphisms, genomic DNA of a subgroup of 83 consecutive patients was obtained from peripheral blood by standard methods. The identification of the polymorphisms was performed following previous procedures.

This study was approved by the Ethics Committee of the hospital, and all patients or relatives gave informed consent.

Statistical Analysis
Descriptive and frequency statistical analyses were obtained and comparisons were made with the use of the SPSS statistical package, version 10.0. Statistical significance for intergroup differences was assessed by the Fisher exact test for categorical variables and the Student t test and ANOVA for continuous variables. CRP values were not normally distributed (Kolmogorov-Smirnov and P-P plot).

Spearman correlation coefficient was used to determine correlations were not normally distributed (Kolmogorov-Smirnov and P-P plot). On admission, all patients underwent a CT within the first 3 hours of stroke onset. No patient with a hypodensity involving >33% of the MCA territory received tPA in this study. CT was repeated after 24 to 48 hours (or earlier when rapid neurological deterioration occurred) to evaluate the presence of hemorrhagic transformations, according to previously published criteria.

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TABLE 1. CRP Level and Presence of Several Risk Factors in the Whole Study Population and in Patients With a Proximal MCA Occlusion

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>All Patients</th>
<th>Proximal MCA Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median CRP, mg/dL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>All Patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Male</td>
<td>0.57</td>
<td>0.52</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>0.53</td>
<td>0.56</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.67</td>
<td>0.41</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.67</td>
<td>0.54</td>
</tr>
<tr>
<td>Dyslipemia</td>
<td>0.59</td>
<td>0.54</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0.65</td>
<td>0.53</td>
</tr>
<tr>
<td>Previous acute MI</td>
<td>0.56</td>
<td>0.59</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>0.99</td>
<td>0.53</td>
</tr>
</tbody>
</table>

*Factors with a value of P<0.05.
TABLE 2. Baseline Factors Associated With Mortality

<table>
<thead>
<tr>
<th></th>
<th>Survival (n=124)</th>
<th>Mortality (n=19)</th>
<th>P</th>
<th>Survival (n=81)</th>
<th>Mortality (n=13)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>51.6</td>
<td>47.4</td>
<td>0.73</td>
<td>51.6</td>
<td>46.9</td>
<td>0.959</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>69.8</td>
<td>76.2</td>
<td>0.014*</td>
<td>68.8</td>
<td>77.3</td>
<td>0.006*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>20.2</td>
<td>20</td>
<td>1</td>
<td>21.3</td>
<td>18.2</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>50.8</td>
<td>68.4</td>
<td>0.153</td>
<td>45.6</td>
<td>76.9</td>
<td>0.036*</td>
</tr>
<tr>
<td>Dyslipemia</td>
<td>15.7</td>
<td>31.6</td>
<td>0.11</td>
<td>12.5</td>
<td>30.8</td>
<td>0.103</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>32</td>
<td>38.9</td>
<td>0.559</td>
<td>33.8</td>
<td>50</td>
<td>0.338</td>
</tr>
<tr>
<td>Previous acute MI</td>
<td>17.2</td>
<td>40</td>
<td>0.076</td>
<td>13.4</td>
<td>41.5</td>
<td>0.023*</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>9.3</td>
<td>37.5</td>
<td>0.006*</td>
<td>7.7</td>
<td>45.5</td>
<td>0.004*</td>
</tr>
<tr>
<td>NIHSS score</td>
<td>17</td>
<td>18</td>
<td>0.035*</td>
<td>18</td>
<td>18</td>
<td>0.094</td>
</tr>
<tr>
<td>CT early signs</td>
<td>47.3</td>
<td>41.7</td>
<td>0.715</td>
<td>61.8</td>
<td>37.5</td>
<td>0.257</td>
</tr>
<tr>
<td>Proximal occlusion</td>
<td>65.3</td>
<td>76.5</td>
<td>0.361</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>153.7</td>
<td>158.4</td>
<td>0.465</td>
<td>152.9</td>
<td>160.4</td>
<td>0.336</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>81.4</td>
<td>80.8</td>
<td>0.882</td>
<td>79.9</td>
<td>84.3</td>
<td>0.276</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>140.5</td>
<td>158.9</td>
<td>0.276</td>
<td>136</td>
<td>172</td>
<td>0.199</td>
</tr>
<tr>
<td>Leukocyte, 109/L</td>
<td>8418</td>
<td>9311</td>
<td>0.198</td>
<td>8855</td>
<td>10 300</td>
<td>0.103</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.39</td>
<td>3.79</td>
<td>0.025*</td>
<td>3.34</td>
<td>3.91</td>
<td>0.012*</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>0.53</td>
<td>0.85</td>
<td>0.002*</td>
<td>0.53</td>
<td>0.81</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Values are median, mean, or percentage, as applicable. MI indicates myocardial infarction; SBP, systolic blood pressure; DBP, diastolic blood pressure.

*Factors with a value of P<0.05.

in patients who died after thrombolysis compared with survivors (P<0.05; Table 2 and Figure 1).

A CRP cut point level (0.77 mg/dL) was obtained from the ROC curve and was used in the multivariate analysis. In the logistic regression model, CRP >0.77 mg/dL (odds ratio [OR]=6.03; 95% CI, 1.97 to 18.39; P=0.002), age >72 years (OR=5.53; 95% CI, 1.59 to 19.15; P=0.007), and a baseline NIHSS score >17 (OR=3.52; 95% CI, 1.14 to 10.86; P=0.029) were the only baseline predictors of mortality. Adjustments for risk factors did not change the results of the multivariate analysis appreciably (for CRP >0.77 mg/dL [OR=5.04; 95% CI, 1.51 to 16.83; P=0.008]; age >72 years [OR=4.45; 95% CI, 1.14 to 17.37; P=0.032]; and baseline NIHSS score >17 [OR=3.01; 95% CI, 0.95 to 9.54; P=0.061]).

Among the 94 patients with a proximal MCA occlusion, a correlation between CRP and mRS was found (r=0.24, P=0.02). This association is mainly because of the high baseline CRP level found among those with the worst scores (0.77 mg/dL). Interestingly, this CRP cut point level (0.77 mg/dL) was also selected from the ROC curve to be included in the multivariate analysis. This point had a sensitivity of 69.2% and a specificity of 75.3% to predict mortality. In the logistic regression model, CRP (OR=8.51; 95% CI, 2.16 to 33.5; P=0.002) and age (OR=6.25; 95% CI, 1.44 to 27.19; P=0.015) were the only baseline predictors of mortality after adjustment for stroke severity. Among patients older than 72 years with CRP >0.77 mg/dL, mortality rates reached 54% (Figure 3).

By the end of tPA infusion, 67 patients (44.7%) showed a complete or partial recanalization of the occluded artery. Among those who recanalized, the 9 patients who died (13.4%) had higher CRP level than those who remained alive (0.87 versus 0.49 mg/dL; P=0.029). In regard to proximal occlusions, 52 patients (50.5%) showed a complete or partial recanalization, and CRP-survival association was even stronger (0.89 versus 0.41 mg/dL; P=0.007), as shown in Figure 4.

Analyzing CRP influence on reperfusion during the available 24-hour sonographic follow-ups, we observed that patients with a persistent MCA occlusion (n=26; 21.5%) had a slight trend (P=0.2) to have a higher CRP level (0.575 mg/dL) than those (n=95; 78.5%) with a successful recanalization (0.520 mg/dL).

In regard to CRP genotype distribution, we identified 74 wild-type patients (89.2%) for G1059C polymorphism, 8 heterozygous patients (9.6%), and 1 homozygous patient (1.2%). Both polymorphisms were in Hardy-Weinberg equilibrium (P=0.397 and P=0.687, respectively). No association was found among these 2 polymorphisms and demographic data, stroke risk factors, hemorrhagic transformations, or recanalization rates (data not shown). G1059C and C1444T polymorphisms were not associated with the levels of
CRP at arrival (wild-type=0.55 mg/dL, heterozygous=0.69 mg/dL, homozygous=1.12 mg/dL [P=0.535]; and wild-type=0.59 mg/dL, heterozygous=0.44 mg/dL, and homozygous=0.67 mg/dL [P=0.930], respectively). Moreover, mortality rates were similar regarding G1059C and C1444T genotype distribution (wild-type=14.5%, heterozygous=25%, and homozygous=0% [P=0.67]; and wild-type=15.4%, heterozygous=14.8%, and homozygous=16.7% [P=0.993], respectively).

Discussion

This study demonstrates for the first time that admission (<3-hour) high-sensitivity CRP level predicts mortality after tPA treatment for stroke patients, adding prognostic information to classic risk factors. Moreover, we found that, unfortunately, very early tPA-induced recanalization of the occluded brain vessel does not ameliorate the negative prognostic impact of elevated CRP. This is of clinical importance because CRP is a powerful marker that may be

Figure 1. Baseline CRP levels in relation to mortality at follow-up among all tPA-treated patients (A; P=0.002) and among those with proximal MCA occlusions (B; P=0.001). Dashed line indicates CRP=0.77 mg/dL, which is the value that better discriminates between survival and death.

Figure 2. CRP associations with outcome among those patients with a proximal MCA occlusion. A, CRP distribution according to mRS scores. B, Mortality rates by CRP quartiles.
used as a point-of-care tool for the risk stratification of stroke patients as candidates to receive tPA.

Stroke patients with a high CRP level should receive particular attention because they have unacceptably high mortality rates. A recent study indicates that the predictive value of CRP for future cardiovascular events is linear across a full range of values. Most importantly, these data demonstrate that very high (\(\geq 1 \text{ mg/dL}\)) levels of CRP provide important prognostic information. This is important because there is no evidence that unusually high values represent false-positive findings. To the contrary, these data indicate that there is considerable predictive value of CRP levels beyond the ranges suggested by the recent Centers for Disease Control and Prevention/American Heart Association guidelines for use of CRP.

In the present study we failed to demonstrate that poor outcome associated with high CRP level is because of any of the studied polymorphisms. Because G1059C and C1444T polymorphisms do not explain the individual variability of CRP among our group of stroke patients, other factors or different genetic background might be responsible for the high CRP levels associated with stroke mortality after thrombolysis. In fact, new functional CRP gene variants have been described recently.

Our observation that stroke patients with high levels of CRP are at very high risk of death is consistent with the hypothesis that CRP may have direct arterial effects. In fact, mice transgenic for human CRP have increased rates of arterial thrombosis compared with wild-type mice that minimally express CRP. Those findings indicate a cause-and-effect relationship for CRP in vascular thrombosis and occlusion. Furthermore, CRP was reported to directly increase tissue factor from peripheral blood monocytes and endothelial cells and to induce plasminogen activator inhibitor-1 expression and activity in human endothelial cells. Thus, CRP might alter thrombosis and fibrinolysis in a manner that makes tPA-induced reperfusion difficult or favors vessel reocclusion. In fact, a slight trend toward higher CRP level among those patients without a recanalization of the MCA has been identified in this study.

In the cardiology field, a large study of 1042 consecutive patients with non–ST elevation acute coronary syndrome treated with an aggressive revascularization strategy confirmed CRP as a strong independent predictor of mortality. In that study, patients with CRP \(\geq 1 \text{ mg/dL}\) had a 4-fold increase in in-hospital and long-term mortality. Interestingly, and in a manner similar to our study, this report demonstrated that very early revascularization does not ameliorate the negative prognostic impact of elevated CRP. Therefore, CRP appears to be a marker that adds prognostic information to the validated Thrombolysis in Myocardial Infarction (TIMI) risk score, and others suggest that both might be used together for enhanced risk stratification in the setting of acute coronary syndromes.

Together with high CRP, advanced age is another independent predictor of mortality after administration of tPA. The risk of death after tPA reached 54% in patients older than 72 years who were in the highest CRP quartile. Therefore, both factors (age and CRP) might negatively interact. In fact, it has been shown that CRP is a strong but nonspecific risk factor for fatal stroke in older persons. Although 1 study has demonstrated that tPA is a safe and effective treatment among older stroke patients, the European Agency for the Evaluation of Medicinal Products has only approved the treatment for those aged <80 years. Therefore, age is still a concern that might be overcome by adding biomarkers, such as CRP, if tPA is given to older patients.

**Study Limitations**

In this study we recruited consecutive, unselected tPA-treated patients with involvement of the MCA territory. Therefore,
we believe that our findings may be extended to all strokes subtypes, but this point needs further confirmation.

We did not serially repeat measurements of CRP. Although others have reported that CRP measured in non–tPA-treated stroke patients between 12 and 24 hours predicted poor outcome better than in those measured within 12 hours, we believe that to be of clinical relevance this measurement needs to be made before tPA administration.

Because the relatively small number of deceased patients in this study precludes formation of subgroups for a more detailed mortality analysis, this should be addressed in a future and larger study.

Our study demonstrates increased mortality among stroke patients treated with tPA who have high CRP levels, but we did not assess whether these patients have higher mortality rates than similar non–tPA-treated patients with high CRP levels. The great limitation in confirming this finding is the fact that currently it is impossible, from a logistic and ethical point of view, to get blood samples within 3 hours of stroke onset from patients identical to those who receive tPA to serve as matched controls.

In conclusion, our study supports that CRP is a useful marker and probably a factor in the pathogenesis of stroke-related mortality. Therefore, measurement of CRP may help in the future to predict outcome among candidates for thrombolyis. The reasons and mechanisms for interindividual differences in CRP level after stroke need to be clarified.

Acknowledgments

This study was supported by a grant from the Spanish government (FIS 02/0773) and by the Spanish neurological diseases network (CIEN). This work is part of the GENO-tPA study conducted by the Proyecto Ictus Research Group to test genetic markers of safety and efficacy among stroke patients receiving tPA.

References

Poststroke C-Reactive Protein Is a Powerful Prognostic Tool Among Candidates for Thrombolysis
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