Background and Purpose—In acute stroke, Iron (Fe) may amplify reperfusion injury by catalyzing the conversion of superoxide and hydrogen peroxide into highly reactive radicals. Transferrin (Tf) is the main protein regulating Fe homeostasis, whereas Ceruloplasmin (CP) is a circulating ferroxidase enzyme able to oxidize ferrous ions to less toxic ferric forms. This study aims at investigating whether CP, Copper (Cu), Tf, and Fe play a role in the pathophysiology of acute stroke.

Methods—We enrolled 35 acute stroke patients and 44 controls. All patients underwent: neurological examination assessed by National Institutes of Health Stroke Scale (NIHSS), ultrasound evaluation of carotid atherosclerosis, brain MRI to quantify ischemic lesion volume and measurement of serum levels of CP, Cu, Tf, Fe, hydro-peroxides, and Total plasmatic antioxidant capacity.

Results—In patients, NIHSS scores were associated with Tf ($r = -0.48, P = 0.004$), hydro-peroxides ($r = 0.34, P = 0.046$), CP ($r = 0.43, P = 0.012$), and lesion volume ($r = 0.50, P = 0.004$). Lesion volume was inversely associated with Tf ($r = -0.44, P = 0.012$). CP and hydro-peroxides were also largely related ($r = 0.81, P < 0.001$). The model multiple $R$ was 0.57, resulting in a 32.5% of explained NIHSS variance with Tf accounting for 23.4% and CP for 9.1%.

Conclusions—CP and Tf levels are representative of clinical status in acute stroke patients. Our findings suggest a protective role of Tf in acute stroke and a possible ambivalent role of CP. (Stoke. 2009;40:1282-1288.)

Key Words: transferrin ▪ ceruloplasmin ▪ copper ▪ iron ▪ acute stroke

A sudden artery occlusion inducing acute cerebral ischemia may be resolved by vessel recanalization, either spontaneous or induced by thrombolysis. Although this reperfusion phase is crucial for the survival of the neurons neighboring the ischemic core, it may also be associated with lipid peroxidation, free radicals production, and glutamatergic excitotoxicity.1 This phenomenon is defined as reperfusion injury. A variety of agents may attenuate neuronal reperfusion injury in animal models, but none of these putative neuroprotectants was confirmed as an effective therapy in clinical trials.2

Ceruloplasmin (CP) is a multicopper enzyme carrying around 95% of circulating copper (Cu). Its main function is to form water, reducing molecular oxygen, and to oxidize ferrous ions to the less toxic ferric form, without releasing reactive oxygen species (ROS). This enzymatic activity confers to CP a relevant antioxidant power and a significant role in iron (Fe) homeostasis because ferrous ions, entering Fenton reactions, take part in the oxidative stress cascade.3

On the other hand, CP was also described to oxidize low-density lipoprotein (LDL),4,5 and elevated CP concentrations were associated with an increased risk of myocardial infarction and stroke.6,7 Similarly, acute-phase proteins were reported to increase in acute stroke,8,9 also in relation to clinical status and LDL oxidation.8–10 Because CP is also an “acute-phase” reactant, it is uncertain whether its relation to vascular diseases is attributable to an oxidative effect on LDL or its role as an inflammation marker.11

Lipid peroxidation may be amplified by nonprotein-bound metals (Cu, Fe): they can promote ROS production and activate the signal transduction system.12 Although in physiological conditions “free” circulating metals are virtually inexistent, during cerebral ischemia local acidosis may induce their release from the transporting proteins. In addition, ROS themselves may dissociate Cu from CP, altering its structure.13 Although fundamental for normal brain activity, Fe may induce neuronal injury by catalyzing the conversion of superoxide and hydrogen peroxide into highly reactive radi-
Iron homeostasis is mainly regulated by transferrin (Tf), a glycoprotein organized in 2 domains containing a Fe-binding site. In serving this function, Tf accepts ferric ions, oxidized by CP, and transports them to other cells where Tf-bound Fe is internalized through endocytosis mediated by Tf-1 receptor. Increased body Fe stores were associated with a poor outcome and an early clinical deterioration after stroke. However, the possible relation between Tf level and clinical outcome in stroke patient has never been explored.

This study aims at investigating whether Cu and Fe as well as their transporting proteins, CP and Tf, play a role in the pathophysiology of acute stroke.

**Patients and Methods**

We selected 57 patients admitted for acute stroke to our Neurological department. At admission, all selected patients underwent brain CT scan to rule out cerebral hemorrhage and a neurological examination, scored according to National Institutes of Health Stroke Scale (NIHSS). Past medical history including vascular risk factors was carefully investigated. All subjects received the best clinical treatment according to the Italian standardized guidelines.

We excluded from the study 6 patients for hyperdense signals at CT scan suggestive of brain hemorrhage. 8 patients for previous history of stroke, 5 pace maker carriers for contraindication to undergo MRI examination, 2 patients for refusing consent, and 1 patient for ongoing treatment with iron.

Thirty-five patients (73.4±14.8 years, 19 men) were enrolled. None of them received thrombolysis because they were admitted later than 3 hours from symptoms onset. All patients underwent:

- Ultrasound examination of neck vessels within 48 hours from stroke onset to assess carotid atherosclerosis (ie, intima-media thickness [IMT], presence of stenotic plaques).
- MRI examination within 5 days from onset to assess ischemic lesion volume.
- Blood sampling within 48 hours from the clinical onset to perform biochemical assays.
- As control group, we retrieved data from 44 subjects (71.1±11.0 years, 24 men) enrolled in a previous study assessing their biochemical profile. This group consisted of elderly volunteers with no clinical evidence or history of neurological or vascular disease.

**Ultrasound Examination of the Cerebral and Neck Vessels**

The carotid and vertebral arteries were studied with color-coded duplex sonography (3 to 9 MHz probe: iU22, Philips Ultrasound). Plaques in the carotid arteries were assessed and defined according to published methods. Ceruloplasmin and transferrin levels were also investigated. Such correlation analysis was performed by immunoturbidimetry assays and iron using Fere, using ABX Pentra from Horiba ABX reagents. For each serum copper and ceruloplasmin pair, we computed the amount of copper not bound to ceruloplasmin (“free” copper) following standard procedures. Briefly, “free” copper (μmol/l) = total Cu–bound Cu, where bound Cu = CP (mg/dL)*0.472. All biochemical measures were automated on a Cobas Mira Plus analyser (Horiba ABX) and performed in duplicate. The neurobiologist performing biochemical assessment was blind to the clinical, radiological, and ultrasound evaluations of the patients.

If present in the hospital database, the values of fibrinogen, white cell count (WCC), reactive c protein (rCP), Erythrocyte sedimentation rate (ESR), and ferritin obtained from the analysis of blood samples taken in the emergency room at admission were collected.

The experimental protocol was approved by the Hospital Ethical Committee, and all patients and controls signed a written informed consent.

**Statistical Analysis**

Because this study aimed at investigating whether Cu, Fe, CP, and Tf have a role in acute stroke, operationally these biochemical values and the neuroradiological measures were correlated to NIHSS scores. The association among biochemical variables and between biochemical, neuroradiological, and neurooncological measures were also investigated. Such correlation analysis was performed by means of Pearson correlation, because all the measures were distributed approximately according to the Gaussian distribution (or easily transformed to fit gaussianity satisfyingly). To increase sensitivity in identifying any association potentially useful to unveil relevant aspects of the pathophysiology of acute stroke, for this first statistical analysis we chose not to adjust probability values for multiple testing. Because such approach could lead to false-positive findings (alpha inflation), (1) exact t-coefficient and probability values were reported to allow the readers to judge the extent of each association, (2) the successive multiple regression analysis was more conservative, indicating which measures were more probably independent markers of stroke severity.

Multiple regression analysis was performed to identify the variables that could likely account for NIHSS variability. To limit the occurrence of Type I errors (false-positive findings), and to avoid type III errors (the effect of a factor is significant but in the opposite direction respect to the true one), only the variables that resulted associated bivariately to the clinical status were entered in the regression analysis. The main analysis was performed with NIHSS as dependent variable and Hydro-peroxides, CP, Tf and Lesion volume as independent variables. In such a way the Case-To-Variables,
resulting in 35/4 = 9, was adequate. Although some variables, not correlated bivariately to NIHSS, could have accounted for a significant portion of NIHSS variability, the sample size of our study did not allow to address this point without increasing the risk of statistical errors. Because NIHSS lacks of a clearly defined unit measure and, strictly, should not be analyzed by means of parametric model, an ordinal regression model was also applied. However, the results were consistent with the parametric model, thus we chose to highlight the parametric findings for their higher simplicity and comparability with other studies.

**Results**

Table 1 summarizes patient clinical, radiological, and demographic characteristics. Because patients and controls were not individually matched for age and gender, slight nonsignificant differences occurred (P > 0.4). Table 2 reports the comparison of the biochemical variables between patients and controls, indicating differences with a large effect size (according to Cohen conventions) in Tf, hydro-peroxides and Cu, and with a medium effect size in terms of Fe.

**Patients**

CP correlated with hydro-peroxides (r = 0.81, P < 0.001) and with ferritin (r = 0.53, P = 0.042; in a subsample of 15 patients). Cu was strictly correlated to CP (r = 0.91, P < 0.001) and to hydroperoxides (r = 0.68, P < 0.001). As

### Table 1. Patients’ Clinical, Radiological, and Demographic Characteristics

<table>
<thead>
<tr>
<th>Vascular Risk Factors, n (%)</th>
<th>Patients (n=35)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male (%)</td>
<td>17 (49%)</td>
<td></td>
</tr>
<tr>
<td>Age, Mean (SD)</td>
<td>73.4 (14.8)</td>
<td></td>
</tr>
<tr>
<td>NIHSS, Mean (SD)</td>
<td>6.9 (3.9)</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>5 (14%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>27 (77%)</td>
<td></td>
</tr>
<tr>
<td>Cardiopathy Atrial</td>
<td>14 (40%)</td>
<td></td>
</tr>
</tbody>
</table>

**Inflammation markers (reference range)**

| Erythrocyte sedimentation rate (mm/hr) [27 pt] | 26.7 ± 9.7 (< 30) |
| Reactive C protein (mg/dL) [16 pt]            | 2.5 ± 6.6 (<0.5)  |

**MRI**

<table>
<thead>
<tr>
<th>Main vascular territory</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>VBA</td>
<td>3</td>
</tr>
<tr>
<td>MCA</td>
<td>26</td>
</tr>
<tr>
<td>ACA</td>
<td>2</td>
</tr>
</tbody>
</table>

**Carotid Ultrasound**

<table>
<thead>
<tr>
<th>Lesion Site n</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Stenosis</td>
<td>27 (77%)</td>
</tr>
<tr>
<td>Mild Stenosis</td>
<td>7 (20%)</td>
</tr>
<tr>
<td>Moderate Stenosis</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Severe Stenosis</td>
<td>4 (11%)</td>
</tr>
</tbody>
</table>

**Plaque index n (%)**

<table>
<thead>
<tr>
<th>IMT (mm) Mean (SD)</th>
<th>Cortical 0.84 (0.18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion Site n</td>
<td>Cortico-Subcortical 20</td>
</tr>
<tr>
<td></td>
<td>Subcortical 13</td>
</tr>
</tbody>
</table>

**Table 2. Biochemical Parameters in Stroke Patients and Controls**

<table>
<thead>
<tr>
<th>Biochemical Parameter</th>
<th>Stroke Patients (n=35), Mean (SD)</th>
<th>Reference Range</th>
<th>Controls (n=44), Mean (SD)</th>
<th>t (73)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper, µmol/L</td>
<td>14.69 (2.30)</td>
<td>11–24.4</td>
<td>12.61 (2.52)</td>
<td>-3.723</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ceruloplasmin, mg/dL</td>
<td>29.20 (8.59)</td>
<td>20–60</td>
<td>26.55 (4.70)</td>
<td>-1.582</td>
<td>0.118</td>
</tr>
<tr>
<td>‘free’ Copper, µmol/L</td>
<td>0.82 (2.26)</td>
<td>0–1.6</td>
<td>0.20 (2.66)</td>
<td>-1.023</td>
<td>0.310</td>
</tr>
<tr>
<td>Iron, µmol/L</td>
<td>11.26 (5.12)</td>
<td>6.6–29.4</td>
<td>13.72 (4.28)</td>
<td>2.242</td>
<td>0.028</td>
</tr>
<tr>
<td>Ferritin, pmol/L [15 pt]</td>
<td>537.7 (557.7)</td>
<td>33.7–561.8</td>
<td>. . .</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Transferrin, g/L</td>
<td>2.16 (0.37)</td>
<td>2–3.6</td>
<td>2.68 (0.40)</td>
<td>5.828</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hydroperoxides, UCARR</td>
<td>687.9 (19.84)</td>
<td>230–310</td>
<td>299.77 (68.50)</td>
<td>-12.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total plasmatic antioxidant capacity, mmol/L</td>
<td>1.41 (0.13)</td>
<td>1.3–1.8</td>
<td>1.39 (0.15)</td>
<td>-0.718</td>
<td>0.475</td>
</tr>
<tr>
<td>CP/Tf Ratio</td>
<td>4.64 (1.25)</td>
<td>. . .</td>
<td>3.39 (0.51)</td>
<td>5.406</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

pt indicates patients; CP/Tf Ratio, ratio between CP and Tf values after log transformation.
Table 3. NIHSS as Function of Demographic and Biochemical Measures: Simple and Multiple Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>Simple Linear Regression</th>
<th>Multiple Linear Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
</tr>
<tr>
<td>Age, years</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Gender, M vs F</td>
<td>1.24</td>
<td>1.36</td>
</tr>
<tr>
<td>Copper, μmol/L</td>
<td>0.51</td>
<td>0.29</td>
</tr>
<tr>
<td>Ceruloplasmin, mg/dL</td>
<td>0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>'free' Copper, μmol/L</td>
<td>−0.83</td>
<td>0.28</td>
</tr>
<tr>
<td>Iron, μmol/L</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Ferritin, pmol/L</td>
<td>0.003</td>
<td>0.004</td>
</tr>
<tr>
<td>Transferin, g/L</td>
<td>−5.09</td>
<td>1.63</td>
</tr>
<tr>
<td>Hydroperoxides, UCARR</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Total plasmatic antioxidant capacity, mmol/L</td>
<td>−2.42</td>
<td>5.21</td>
</tr>
</tbody>
</table>

*15 patients.

expected, “free” Cu was inversely correlated with CP ($r = −0.628, P < 0.001$). Transferrin was positively associated with Fe ($r = 0.39, P = 0.022$) and negatively with ferritin ($r = −0.51, P = 0.053$). Fe was also correlated with ferritin ($r = −0.65, P = 0.008$). TAS resulted correlated to Cu ($r = 0.39, P = 0.021$).

The relationship between NIHSS and biochemical measures are summarized in Table 3, where age and gender were also reported to take into account basic demographic characteristics.

Lesion volume presented a significant correlation with NIHSS ($r = 0.50, P = 0.004$) as well as, inversely, with Tf ($r = −0.44, P = 0.012$). No evidence of association between CP, Tf, Cu, “free” Cu, Fe, Ferritin, TAS, as well as NIHSS, with inflammation markers was found (all $P > 0.2$, apart from CP and fibrinogen: $r = 0.37, P = 0.061$). Conversely, markers of inflammation were associated with each other, being the lowest correlation between ESR and WCC ($r = 0.38, P = 0.054$) and the highest between fibrinogen and rCP ($r = 0.73, P = 0.005$).

Neurosonological parameters did not correlate with either biological measures or NIHSS ($r$ consistently lower than 0.25, $P > 0.15$). The analysis on the possible relation between biochemical and inflammation parameters and vascular risk factors showed a significant association between Tf and cardiopathy ($P = 0.001$) and between fibrinogen and hypertension ($P = 0.049$).

Multiple Regression Analysis

To assess the significance of the biological variables correlating with the NIHSS in explaining patients’ clinical status, a multiple regression analysis was performed taking into account Hydro-peroxides, CP, “free” Cu, and Tf as the independent variables and NIHSS as the dependent one. Age and gender were added as forced terms to take into account their possible effect. This model showed that Tf and CP were the only measures associated to the clinical status (Table 3). CP accounted for a significant proportion of NIHSS variance after taking into account hydroperoxides (7%), whereas hydroperoxides did not add a significant contribution after taking into account CP (0%). Transferrin alone accounted for the 23.4% of NIHSS variance whereas CP contribution was 9.1%. Thus the model multiple R was 0.57, resulting in a 32.5% of explained NIHSS variance. A unitary increase of TF is estimated to reduce NIHSS of −4.47 point, whereas a unitary increase of CP is estimated to increase NIHSS of 0.17.

As indicated in the Statistical Analysis paragraph, to test the robustness of our findings, the relationship between NIHSS as dependent variable and Tf and CP as independent variables was also analyzed by means of an ordinal regression (PLUM algorithm in SPSS syntax). Because the frequency distribution of NIHSS suggested that lower values were more probable (confirmed by a positive value of skewness), negative log-log link function was chosen. The ordinal regression was fairly consistent with the parametric regression (Tf: estimate = −1.327, SE = 0.566, $P = 0.019$; CP: estimate = 0.505, SE = 0.233, $P = 0.028$). Given the opposite associations between NIHSS-Tf and NIHSS-CP, we deemed it useful to compute their ratio to verify whether their relative weight could be a unique and efficient marker of clinical status. The mean values of the ratio between CP and Tf after log transformation (CP/Tf ratio) are reported in Table 2. When the CP/Tf ratio was taken into account in this regression model, this measure was the only independent factor to enter the regression model, excluding both CP and Tf. Multiple R was similar to that obtained entering both variables ($R = 0.58$; Figure).

When lesion volume was added as covariate to adjust the relationship between clinical status and biological markers, the significant effect of the CP/Tf ratio was not affected ($P = 0.002$). The combination of lesion extension and relationship between biological markers was able to account for about 50% of the clinical status ($R^2 = 0.47$).

Discussion

The main result of this study is that serum CP and Tf levels are representative of clinical status severity in acute stroke patients.

Transferrin had a stronger relation to clinical status than CP and, unlike CP, it correlated inversely with the ischemic
lesion volume. These findings suggest that Tf may play a protective role in the early phases of stroke progression, limiting ischemic damage extension. This possible positive effect is likely attributable to Tf capacity to reduce Fe availability to react with hydroperoxides, binding Fe in concert with CP ferroxidase activity. Fe reacts with hydroperoxides in a Fenton-like reaction, generating the hydroxyl radical, the most toxic oxygen metabolite. The protective action of Tf is also supported by the observation that an efficient CP/Tf system reduces lipid peroxidation. Moreover, apotransferrin was reported to reduce the circulating redox-active Fe, protecting in this way the renal parenchyma against the ischemia-reperfusion injury. Despite these evidences, clinical trials testing the neuroprotective activity of an inhibitor of Fe-dependent perioxidation were stopped for safety concerns, suggesting that Tf might hinder the ischemic damage also with other mechanisms.

In our stroke patients Tf concentration was significantly lower with respect to controls. This finding can be explained by the inhibition of Fe regulating proteins reported at early times of postischemic reperfusion. Moreover, hypoxia-inducible factor–1 mediates an enhancement of Tf receptor transcription. The upregulation of Tf receptor would rise the rate of internalization via endocytosis of the complex Fe/Tf/Tf-receptor-1 during Fe cellular uptake, reducing Tf and Fe serum level. This increase of extra/intracellular Tf cycling might also account for the slight lower serum concentration observed in our patients with respect to controls.

Differently from Tf, serum CP levels were positively related to clinical severity and to hydroperoxide concentrations. In our patients, hydroperoxides had a 2-fold increase with respect to controls, representing a feasible sign of the peroxidation processes occurring during the acute phase of stroke. Ceruloplasmin synthesis might have increased as a defense mechanism in response to hypoxia and to the burst of oxidative damage, in account of its ferroxidase activity. Aceruloplasminemia offers an example of the impact of the CP impairment on brain Fe metabolism: its absence leads to the abnormal deposition of Fe in cells and increased lipid peroxidation, thus triggering oxidative stress and ultimately leading to neurodegeneration. In addition, CP was also reported to contribute to cytoprotection after ischemia-reperfusion injury through nitric oxide (NO) oxidation and nitrite synthesis.

Our analysis indicates that the correlation between CP and hydroperoxides explains only partially the relation between CP and the clinical status in our patients, suggesting that we should consider additional hypotheses. We previously demonstrated a systemic increase of circulating non-CP copper in patients affected by Alzheimer disease. In the present study, although total Cu serum concentrations were significantly higher in patients with respect to controls, systemic “free” Cu did not differ in the 2 populations. Besides, total Cu level resulted largely related to CP concentration. These findings suggest that the systemic total Cu increase observed in our patients mainly corresponds to bound Cu (primarily to CP and secondarily to other binding proteins). However, given the significant interrelation found among total Cu, CP, and hydroperoxide levels, we may speculate that Cu is involved in the redox toxic reactions close to the brain lesion. In fact, in the acid pH taking place at the damaged tissue level, CP can release Cu which, in turn, could react with hydroperoxides further amplifying the ischemic injury. In addition, lipid peroxidation at the ischemic site inducing a local production of hydroxyl radicals may alter CP structure and thus impair its detoxifying ferroxidase activity. Given its role both in Fe efflux and influx also into the brain cells, CP might also increase Fe concentration in the cerebral parenchyma. Moreover, under a condition of limited oxygen supply, CP was reported to be involved in iron release from macrophages. If Fe transported by CP exceeds Tf capability to bind it, this might accumulate and take part to the toxic cascade above described. In this respect, it is worth noting that CP/Tf ratio represented clinical status with a greater accuracy with respect to the parameters considered independently.
The role of CP/Tf system was previously investigated in preeclampsia, a condition associated with an oxidative stress damage. Similarly to our patients, women with severe preeclampsia presented higher levels of lipid peroxidation and serum CP, and lower levels of Tf with respect to pregnant women healthy or with mild preeclampsia.43–44 These findings suggest that CP/Tf system is involved in the oxidative stress generated in different body districts.

Acute phase proteins were reported to increase in relation with clinical outcome from stroke.8,9,10 Because Tf and CP are, respectively, a negative and a positive acute phase protein,45 it cannot be excluded that their relation with stroke severity is mediated by inflammation. Tf and CP might represent just inflammatory markers or, alternatively, although regulated by the release of inflammatory cytokines (eg, IL-6), play a pathogenic role in stroke progression. In our patients, even if the other inflammation markers were largely related to each other, they were not related either to CP and Tf or to the clinical status. This finding is in line with previous observations on reactive C protein consistently reporting that although this sensitive marker of inflammation had a prognostic relevance in long-term stroke outcome, its concentration was not related to clinical status in the acute phase.46,47 Even dismissing a pathogenetic role, CP and Tf strong relation with neurological status candidates these protein and their ratio as markers of clinical severity. Future studies are necessary to test their prognostic value and reliability to predict clinical progression in the acute phase of stroke.

Finally, we also investigated in our patients whether Tf, Fe, CP, and Cu concentrations were related to carotid atherosclerosis. Although we did not find any relation, their effect on atherosclerosis cannot be excluded. As described above, stroke may have altered their concentrations, hiding their possible role in plaque formation.

The main limitation in interpreting the results of our study is that CP, Cu, Fe, and Tf levels were measured in the systemic circulation. In physiological conditions, although Tf may cross the blood–brain barrier via endocitosis, both CP and Tf do not diffuse through it because of their high molecular weight. However, the blood–brain barrier becomes permeable to larger molecular sizes within 24 hours from the initial ischemic damage, resulting in a compound exchange between serum and extracellular neuronal space.48 In this condition even larger molecules, like CP and Tf, may diffuse across the disrupted blood–brain barrier and take part to the pathological reperfusion cascade. Besides, because circulating CP and cerebral isofoms present the same gene promoters, CP serum concentrations might reflect its cerebrospinal fluid levels.49 On the other hand, performing these biochemical measurements in the serum may actually represent an advantage in prospect of testing CP and Tf as prognostic factors. The small sample of patients and the lack of a qualitative assessment of CP and Tf biochemical status represent the other main limitations of this study.

Taken together, our findings support the hypothesis that Tf may hinder the oxidative damage induced by cerebral ischemia, although the role played by CP remains unclear. Future studies are needed to confirm our data in a larger sample of patients and to better clarify the biological mechanisms subtending these findings.

Acknowledgments
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Disclosures
None.

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Stroke. 2009;40:1282-1288; originally published online February 19, 2009;
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