Inflammation-Mediated Damage in Progressing Lacunar Infarctions

A Potential Therapeutic Target

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Background and Purpose—The mechanisms underlying neurological deterioration in patients with lacunar infarction are not completely understood. In this study, we sought to investigate the role of proinflammatory molecules in the early worsening and outcome of acute lacunar stroke.

Methods—We performed a secondary analysis of 113 consecutive patients with lacunar infarction included within the first 24 hours of the onset of symptoms in a previous study aimed at investigating clinical and biochemical factors of early neurological deterioration (END). END was defined as a fall of ≥1 points in the motor items of Canadian Stroke Scale between inclusion and 48 hours. Poor outcome at 3 months was considered death or Barthel Index <85. Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and intercellular adhesion molecule-1 (ICAM-1) were determined by enzyme-linked immunoabsorbent assay in blood samples obtained on admission.

Results—END was recorded in 27 patients (23.9%); poor outcome was noted in 26 (23%). Median (quartiles) concentrations in plasma of TNF-α [16.5 pg/mL (13.7 and 21.2 pg/mL) versus 7.5 pg/mL (6.2 and 9.0 pg/mL)], IL-6 [28.8 pg/mL (22.5 and 35.7 pg/mL) versus 11.5 pg/mL (8.5 and 16.2 pg/mL)], and ICAM-1 [285 pg/mL (219 and 315 pg/mL) versus 158 pg/mL (137 and 187 pg/mL)] were significantly higher in patients who had END than in those with nonprogressing strokes ($P<0.001$). Significant differences were also observed between patients with poor and good outcome at 3 months. Logistic regression analysis after adjustment for potential confounders showed that TNF-α >14 pg/mL and ICAM-1 >208 pg/mL were independently associated with both END (OR, 511; 95% CI, 17 to 4937; $P<0.001$; and OR, 315; 95% CI, 17 to 5748; $P<0.001$, respectively) and poor outcome at 3 months (OR, 3.0; 95% CI, 1.0 to 8.5; $P=0.042$; and OR, 4.2; 95% CI, 1.3 to 13.6; $P<0.015$, respectively).

Conclusions—High concentrations of inflammatory markers in blood are associated with END and poor functional outcome in lacunar infarctions. These findings suggest that inflammation contributes to brain injury in lacunar stroke. **(*Stroke*. 2002;33:982-987.)**

Key Words: cytokines ■ inflammation ■ lacunar infarction ■ stroke outcome

Approximately 30% of all ischemic stroke patients have lacunar infarctions, and between 25% and 35% of them suffer neurological deterioration within the first few hours of the onset of symptoms and have a worse prognosis.[1–8] Although some clinical factors have been associated with progressing lacunar stroke,[4–7] the mechanisms involved in this progression have not been clearly established. Clinical and experimental research over the last few years has shown that inflammatory mechanisms participate in stroke-induced brain damage.[9–11] Increased levels of cytokines such as interleukin (IL)-1, tumor necrosis factor-α (TNF-α), and IL-6,[12–15] as well as adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1),[16–18] have been observed after experimental brain ischemia. Clinical studies have reported increased levels of proinflammatory cytokines[19–22] and adhesion molecules[23–25] in the peripheral blood and cerebrospinal fluid (CSF) of patients with ischemic stroke. High IL-6 concentrations in CSF and plasma have been associated with larger infarct size, neurological deterioration, and poor outcome independently of the stroke subtype.[19,22,26–29]

However, although accumulating evidence suggests that inflammatory-mediated damage plays a role in brain ischemia, it remains unclear whether inflammation also intervenes in lacunar stroke progression and outcome. In this study, we sought to investigate the potential association between high

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concentrations of proinflammatory molecules in blood and the poor prognosis of lacunar infarctions.

Subjects and Methods

We performed a secondary analysis of 113 consecutive patients (mean age, 69.7±9.3 years; 57% male) with lacunar infarction included within the first 24 hours of the onset of symptoms in a previous study aimed at investigating clinical and biochemical factors of early neurological deterioration (END) in lacunar stroke.[30] The control group included 43 healthy subjects (60.4% male; age, 55±17 years) without neurological disorders or inflammatory diseases. Patients with inflammatory or infectious diseases, cancer, hematological diseases, and severe renal and liver failure, as well as those who were under treatment with antiinflammatory drugs, were excluded. The study was approved by the ethics committees of both hospitals, and informed consent was obtained from patients or their relatives. A detailed description of the protocol has been published elsewhere.[30] In summary, it included a medical history with recording of potential stroke risk factors, clinical examination, blood and coagulation tests, 12-lead ECG, chest radiography, arterial supraaortic trunk examination, and transcranial Doppler. Cranial CT was carried out at admission, and CT or MRI was repeated between the third and seventh days of the onset of symptoms. The second neuroimaging study was taken as the gold standard for the identification of lacunar infarction. Evaluation of all CT scans and MRI was carried out by the same neuroradiologist who was blinded to the clinical and biochemical results. Topographic classification of lacunar infarctions was assessed according to our previously defined criteria.[30] Lacunar stroke was diagnosed when the patient had 1 of the clinical lacunar syndromes[1,31] lasting >24 hours, no evidence of cortical dysfunction, and a normal or deep focal infarction with a diameter ≤15 mm in an appropriate location visualized by CT scan and/or MRI.

Stroke severity was quantified by an experienced neurologist using the Canadian Stroke Scale (CSS)[32] at admission and 48 hours after inclusion. The CSS measures level of consciousness (alert=3, drowsy=1.5); speech (normal=1, expressive deficit=0.5, receptive deficit=0); orientation (oriented=1, disoriented or not applicable=0); facial paresis (none=0.5, present=0); and weakness in arm, hand, and leg (none=1.5, mild=1, significant=0.5, total=0, scored individually for each item), with a total score ranging from 1.5 (maximum deficit) to 10 (absence of deficit). A fall of ≥1 points in the motor items of the CSS between admission and 48 hours was considered neurological deterioration. In accordance with the diagnostic criteria for lacunar stroke, only changes in the motor items of CSS were considered. The Barthel Index was used to evaluate the functional condition of patients at 3 months. Poor outcome was defined as death or a Barthel Index score of <85, which is the level at which patients report that they need help performing day-to-day activities, with a sensitivity of 95% and specificity of >80%.[33]

Laboratory Tests

On admission, blood samples were collected in tubes with potassium edetate, centrifuged at 3000*g* for 5 minutes, and immediately frozen and stored at −80°C. Plasma IL-6, TNF-α, and ICAM-1 levels were measured with commercially available quantitative sandwich enzyme-linked immunoabsorbent assay kits (Quantikine; R&D Systems). These determinations were blinded to clinical and radiological data. Plasma glutamate and GABA levels were quantified by high-performance liquid chromatography as previously described.[30]

Statistical Analysis

Proportions between groups were compared by use of the χ^2 test. Given that proinflammatory molecules were not normally distributed, they were expressed as median (quartiles) and compared between 2 groups by the Mann-Whitney test. The Kruskal-Wallis test was used to compare the inflammatory molecule concentrations of 5 groups of patients with different degrees of improvement or worsening in CSS score between admission and 48 hours (absolute differences: group 1, ≥2; group 2, 1.5 and 1.0; group 3, 0.5, 0, and −0.5; group 4, −1.0 and −1.5; and group 5, ≤2.0).

Spearman's correlation coefficient was used to analyze the association between inflammatory molecules and baseline continuous variables, including age, time from onset to inclusion, CSS score, systolic and diastolic blood pressures, body temperature, hematological and biochemical parameters, and glutamate and GABA concentrations.

Logistic regression analysis was used to determine the importance of the inflammatory markers in END and poor outcome of lacunar stroke. The first models were fitted to assess the adjusted odds ratios (ORs) of END and poor outcome for the proinflammatory molecules. Those clinical variables that reached a value of $P<0.15$ in the bivariate analysis were included as covariates. The parallel kinetic of release between TNF-α and IL-6 probably determines the high correlation that exists between these 2 molecules. This high correlation did not permit us to perform the analysis including TNF-α, IL-6, and ICAM-1 in the same model. Because TNF-α seems to act

TABLE 1. Baseline Clinical Characteristics and Biochemical Parameters

Male sex, n (%)	64 (56.6)
Age, y	69.7±9.2
Mean time from stroke onset to blood sampling, h	10.3±6.9
History of stroke risk factors, n (%)	
Arterial hypertension	61 (54)
Cigarette smoking	27 (23.9)
Alcohol intake (>40 g/d)	27 (23.9)
Atrial fibrillation	13 (11.5)
Diabetes mellitus	35 (31)
Prior stroke or transient ischemic attack	33 (29.2)
Aspirin intake before stroke	15 (13.3)
Clinical characteristics	
CSS on admission	7.8±1.2
Lacunar syndrome on admission, n (%)	
Pure motor hemiparesis	59 (52.2)
Pure sensory stroke	12 (10.6)
Ataxic hemiparesis	5 (4.4)
Dysarthria or clumsy hand	4 (3.5)
Sensory-motor stroke	33 (29.2)
Suspected cause, n (%)	
Large-artery atherosclerosis	15 (13.3)
Cardioembolism	23 (20.4)
Small-vessel disease	72 (63.7)
Undetermined*	3 (2.7)
Biochemistry and vital signs at admission	
Plasma glucose, mg/dL	156±47
Plasma fibrinogen, mg/dL	399±92
Hematocrit, %	39.0±5.8
Leucocyte count, 10^3/mm^3	8.3±1.9
Platelet count, 10^5/mm^3	204±79
Systolic blood pressure, mm Hg	178±26
Diastolic blood pressure, mm Hg	92±14
Body temperature, °C	36.7±0.7

Continuous variables are expressed as mean±SD.

*Coexistence of 2 potential causes of stroke: atrial fibrillation and severe ipsilateral carotid or middle cerebral artery stenosis.

TABLE 2. Median Concentrations of Inflammatory Markers in Patients With Lacunar Infarctions and Control Subjects

	Patients (n=113)	Control Subjects (n=43)	P
IL-6, pg/mL	13.9 (9.2, 23.8)	3.1 (1.3, 4.1)	<0.001
TNF-α, pg/mL	8.2 (6.4, 15.3)	7.0 (5.7, 8.4)	0.001
ICAM-1, pg/mL	187 (172, 223)	167 (140, 207)	0.015

Numbers in parentheses are quartiles.

as the primary "trigger" of the inflammatory cascade,[13] a first analysis was carried out including only plasma TNF-α and ICAM-1 concentrations. Inflammatory markers were included as categorical variables because the cutoffs meant that there was a lack of linearity of the ORs (1=high, 0=low). Cutoff values were calculated by the method described by Robert et al.[34] Because we previously found a relationship between neurotransmitter amino acids and the progression of lacunar infarctions,[30] the odds of END for TNF-α and ICAM-1 were further adjusted for plasma glutamate and GABA concentrations.

Results

The main characteristics of the studied population are summarized in Table 1. Plasma IL-6, TNF-α, and ICAM-1 concentrations were significantly higher in patients with lacunar infarctions than in the control group (Table 2). Similar levels of proinflammatory molecules were found between groups classified by the suspected cause of lacunar stroke, lacunar syndromes, and the presence or absence of the main stroke risk factors such as hypertension, diabetes mellitus, atrial fibrillation, and prior stroke or transient ischemic attack (data not shown). However, significantly lower levels of IL-6 [11.2 pg/mL (7.2 and 13.8 pg/mL) versus 15.8 pg/mL (9.8 and 26.2 pg/mL), $P<0.01$], TNF-α [7.6 pg/mL (6.5 and 9.4 pg/mL) versus 8.3 pg/mL (6.4 and 16.2 pg/mL), $P<0.05$], and ICAM-1 [141 pg/mL (129 and 166) versus 169 pg/mL (143 and 213 pg/mL), $P<0.01$] were found in the 15 patients who were under aspirin treatment at stroke onset compared with those who did not take aspirin.

TNF-α [11.5 pg/mL (7.8 and 16.2 pg/mL) versus 7.6 pg/mL (6.2 and 13.3 pg/mL), $P<0.01$] and ICAM-1 [193 pg/mL (153 and 264 pg/mL) versus 162 pg/mL (138 and 196 pg/mL), $P=0.01$] but not IL-6 concentrations were significantly higher in patients with lacunar infarctions located at the basal ganglia and brainstem than in those with normal CT/MRI or lacunar infarctions located at the white matter.

Plasma concentrations of the proinflammatory molecules did not correlate with age, time from stroke onset to inclusion, baseline CSS score, body temperature, systolic and diastolic blood pressures, hematocrit, platelet and leukocyte count, and fibrinogen and glucose levels (data not shown). However, significant correlations were found between glutamate and GABA levels and concentrations of IL-6 ($r=0.46$ and $r=-0.47$), TNF-α ($r=0.39$ and $r=-0.42$), and ICAM-1 ($r=0.34$ and $r=-0.44$) (all $P<0.001$).

END was recorded in 27 patients (23.9%), and poor outcome at 3 months was found in 26 (23%). Thirteen patients (48%) with END and 13 patients (15%) without END had poor outcome ($P=0.001$). As previously described, baseline clinical and radiological factors associated with subsequent END were history of hypertension, high leukocyte count, and basal ganglia or brainstem location of lacunar infarction, whereas prior treatment with aspirin prevented worsening.[30] Patients with poor outcome showed a significantly higher baseline stroke severity (mean±SD CSS score, 7.2±1.0 versus 8.0±1.2; $P=0.003$), systolic blood pressure (191±26 versus 174±25 mm Hg, $P=0.006$), serum glucose (177±39 versus 150±48 mg/dL, $P=0.009$), and body tem-

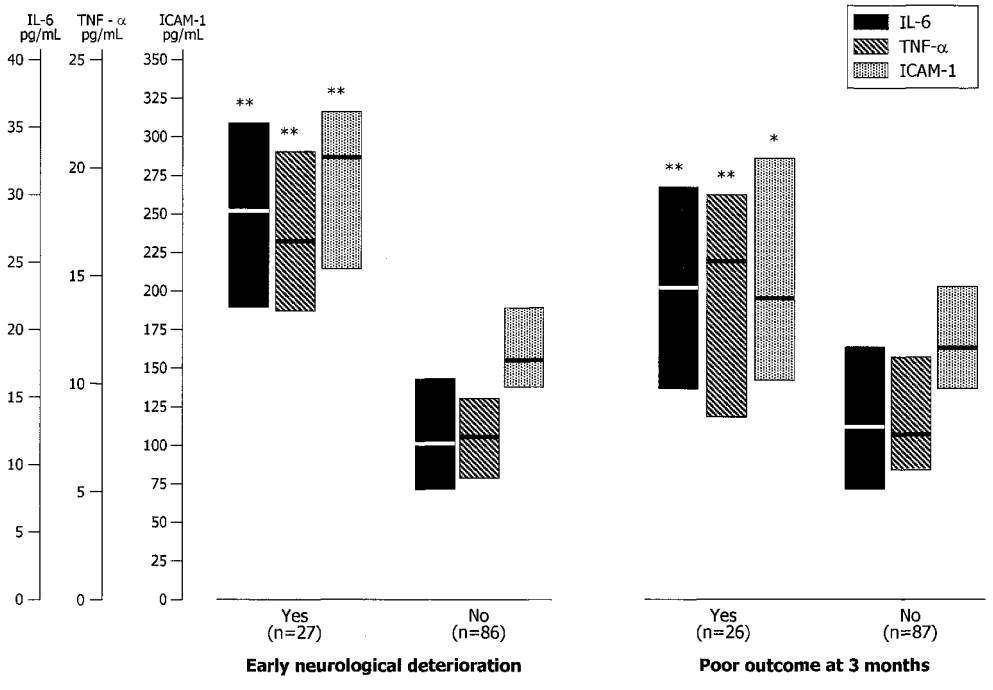

Figure 1. Median values and quartiles (25% and 75%) of plasma inflammatory markers by early clinical course and outcome at 3 months. *$P<0.05$; **$P<0.001$.

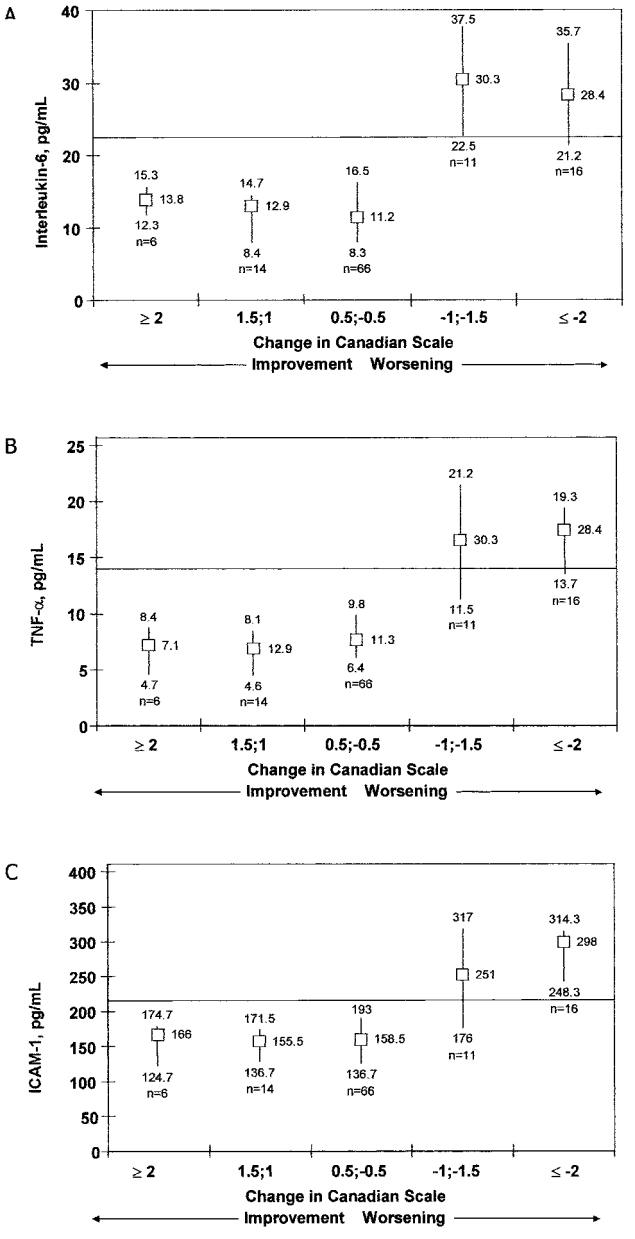

Figure 2. Median values and quartiles of plasma inflammatory molecule levels by absolute difference in CSS score between admission and 48 hours. A, IL-6 concentrations (Kruskal-Wallis test, $P<0.001$); B, TNF-α concentrations (Kruskal-Wallis test, $P<0.001$); C, ICAM-1 concentrations (Kruskal-Wallis test, $P<0.001$). Horizontal lines indicate cutoff values as selected by the method of Robert et al.[34]

perature (37.0±0.7°C versus 36.7±0.6°C, $P=0.013$) compared with those with good outcome.

As shown in Figure 1, median plasma levels of IL-6, TNF-α, and ICAM-1 on admission were significantly higher in patients with END than in patients who remained stable or improved during the first 48 hours. The levels of the inflammatory molecules were also significantly higher in those patients with poor outcome at 3 months. Although the median values of these biochemical compounds were higher in those patients with any degree of END, we did not observe a graded relationship between concentrations of IL-6, TNF-α, and ICAM-1 and the degree of change in the CSS score (Figure 2).

TABLE 3. Adjusted ORs of END for Baseline Clinical, CT, and Biochemical Variables

Variable	OR (95% CI)	*P*
History of hypertension	79 (4.2–1475)	0.003
Leukocyte count, $10^3/mm^3$	1.2 (0.70–2.05)	0.517
Topography on CT*	7.7 (0.6–97)	0.113
TNF-α >14 pg/mL	511 (17–14937)	<0.001
ICAM-1 >208 pg/mL	315 (17–5748)	<0.001

Cutoff values of TNF-α and ICAM-1 were calculated by the method of Robert et al.[34] (see Subjects and Methods).

*Infarct location in basal ganglia or brainstem.

Logistic regression analysis showed that plasma TNF-α >14 pg/mL and ICAM-1 >208 pg/mL were significantly associated with neurological deterioration independently of the history of arterial hypertension, leukocyte count, and infarct location (Table 3). All patients taking aspirin at the onset of stroke had a subsequent nonprogressing course, so the model could not be adjusted for this particular factor. However, the results of the logistic model were not modified after the exclusion of patients under prior treatment with aspirin. The ORs of END for TNF-α and ICAM-1 did not change after adjustment for plasma glutamate and GABA concentrations (Table 4). Plasma TNF-α >14 pg/mL (OR, 3.0; 95% CI, 1.0 to 8.5; $P=0.042$), ICAM-1 >208 pg/mL (OR, 4.2; 95% CI, 1.3 to 13.6; $P<0.001$), and baseline CSS score (OR, 0.48; 95% CI, 0.29 to 0.79; $P=0.004$) were independently associated with poor outcome at 3 months.

Discussion

This study demonstrates in a large series of patients with lacunar infarctions an independent association of high levels of inflammatory molecules in blood with END and poor outcome. The effect of these compounds on END was stronger because the outcome depends particularly on baseline stroke severity. The mechanisms involved in END of lacunar infarctions have not been clearly established, and it seems unlikely that those factors currently accepted as contributing to worsening in other stroke subtypes offer a full explanation in the case of lacunar stroke. Increase in infarct volume has been proposed as the main cause of neurological deterioration,[6,35] a fact that could be explained by a delayed propagation of neuronal death mediated by multiple molec-

TABLE 4. ORs of END for Inflammatory Markers After Adjustment for Serum Glutamate and GABA Concentrations

	OR (95% CI)		
Variables	Model A	Model B	Model C
TNF-α >14 pg/mL	39 (4.6-336)	28 (3.0-266)	37 (1.6-863)
ICAM-1 >208 pg/mL	125 (14.7-1067)	76 (8.1-712)	70 (3.0-1594)
Glutamate >200 μmol/L	...	11 (2.1-59)	...
GABA <240 nmol/L	...	...	184 (11.7-2893)

Model A was not adjusted for neurotransmitters; model B was adjusted for serum glutamate concentrations; model C was adjusted for serum GABA concentrations. Cutoff values were calculated by the method of Robert et al[34] (see Subjects and Methods).

ular and cellular mechanisms such as excitotoxicity and inflammation. We have recently reported that excitotoxicity may play a role in the pathophysiology of progressing lacunar infarctions.[30] High plasma glutamate concentrations and low GABA levels on admission were significantly associated with subsequent neurological worsening. The present findings support the hypothesis that inflammation may also have an important role in the progression of lacunar infarctions.

TNF-α promotes the expression of adhesion molecules such as ICAM-1 on the endothelium, facilitating leukocyte adherence and migration from capillaries into the brain, microvessel occlusion, and subsequently a progressive reduction in blood flow.[13,36] The accumulation of polymorphonuclear neutrophil leukocytes in the ischemic area as a result of the inflammatory process has been proved in a few clinical observations with brain scintigraphy or brain SPECT with labeled leukocytes.[37,38] High levels of TNF-α have been detected as soon as 15 hours in brain samples of stroke victims, peaking during days 2 and 3,[39] and plasma determinations after acute stroke have demonstrated an early activation of ICAM-1, which peaks within 24 hours of cerebral ischemia.[23,25] These findings are in accordance with our results because we found increased TNF-α and ICAM-1 levels within 24 hours (mean, 11 hours) of the onset of lacunar stroke. High levels of adhesion molecules may reflect a prior condition of chronic endothelial activation secondary to risk factors for atherosclerosis[40] such as hypertension, which was significantly more frequent in our patients who had END. In this study, however, high ICAM-1 levels remained independently associated with subsequent END after controlling for the history of hypertension in the logistic analysis.

An interesting finding in this study is that inflammatory molecules contributed to END after adjustment for glutamate and GABA concentrations in blood and that the ORs for TNF-α and ICAM-1 were even higher than that for glutamate concentrations (see Table 4). These results suggest that inflammation may have an additional and stronger role than excitotoxicity in END of lacunar infarctions.[30] On the other hand, inflammatory and excitatory mechanisms might cooperate in the progression of lacunar stroke because we have found a significant correlation between glutamate or GABA concentration and inflammatory markers in blood. Furthermore, as occurred with amino acid concentrations,[30] we have observed higher levels of inflammatory molecules in patients with lacunar infarctions located in basal ganglia and brainstem than in those with white matter infarctions, so excitotoxicity and inflammation might represent sequential and interacting processes in the progression of lacunar stroke, particularly in brain areas with a high density of glutamatergic neurons.[41] This hypothesis is supported by experimental data suggesting that cytokines influence glutamate receptor-mediated excitotoxicity. The addition of TNF-α to human brain cell cultures of embryonic neurons previously treated with glutamate resulted in an increase in neuronal loss, which was blocked with anti–TNF-α antibodies and with the addition of NMDA-receptor antagonists.[42] Other experiments have shown that the infusion of a low dose of IL-1 receptor antagonist causes a 71% reduction in the volume of infarction induced by NMDA-receptor activation,[43] whereas the administration of IL-10, which has been related to neuroprotective actions, results in a reduction in glutamate-induced neuronal death.[44]

One of the major questions in our study is whether increased inflammatory molecules in blood are the expression of brain ischemia or originate as a result of the acute-phase reaction or systemic causes. Several facts support the idea that plasma levels of IL-6, TNF-α, and ICAM-1 within the first 24 hours of acute stroke reflect the total release of these molecules in the ischemic brain tissue. Although in this work we have not performed CSF determinations, previous studies have shown a good correlation between CSF and plasma levels of inflammatory molecules.[22,27] As we previously reported in this series of patients,[30] END and non-END groups did not show differences with respect to cardiovascular risk factors, stroke severity, pathophysiology, biochemical parameters, and vital signs evaluated at the moment in which blood samples were taken, so we cannot attribute the differences in levels of inflammatory molecules to a different acute-phase response or a distinct prior comorbidity. Although potential asymptomatic infections were not excluded by appropriate serological investigations, fever and other medical conditions were similar in frequency in both groups. However, a possible participation of systemic causes or an acute-phase reaction in the serum levels of inflammatory molecules cannot be totally ruled out.

A further point of interest is that prior treatment with aspirin was associated with lower levels of proinflammatory molecules in blood and with a lack of END in lacunar stroke. Some clinical studies have demonstrated that aspirin may reduce the severity and size of cerebral infarction, as well as the frequency of END.[45,46] Recently, a neuroprotective effect of aspirin has been proposed because low doses of aspirin, at the antiplatelet range, have been related to inhibition of glutamate release in both clinical and experimental conditions of focal cerebral ischemia.[47,48] Furthermore, high doses of aspirin may inhibit the activation of necrosis factor-κB and, in turn, the inflammatory cytokines.[49] However, a confounder effect of aspirin in this study may be reasonably ruled out because the association between high levels of proinflammatory molecules and END or poor outcome remained after exclusion of patients taking aspirin.

Although our work has only partially evaluated the effects of the inflammatory cascade, the present findings suggest that inflammation contributes to the END and poor prognosis of lacunar infarctions. Further studies are needed to confirm these promising results, which open new therapeutic avenues in lacunar infarctions.

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