Leukocyte Aggregation in Patients With a Previous Cerebral Ischemic Event

Mauro Silvestrini, MD; Antonio Pietroiusti, MD; Andrea Magrini, MD; Maria Matteis, MD; Sandro Carta, MD; Giorgio Bernardi, MD; Alberto Galante, MD

Background and Purpose The role of leukocyte aggregation in ischemic stroke is controversial. In this study we investigated this hemorheologic alteration in patients at risk for stroke.

Methods Leukocyte aggregation was evaluated with the leukergy test in 61 patients with a recent cerebral ischemic event and in 61 control subjects.

Results In patients leukocyte aggregation was significantly higher than in control subjects (3.8±2.4% versus 2.6±2.2%; P=.01). In control subjects, the presence of vascular risk factors was associated with values of aggregation similar to those observed in patients.

Conclusions These findings suggest an association between altered leukocyte aggregation and cerebrovascular disease. Further investigations are needed to evaluate whether this hemorheologic alteration can be considered a marker of increased risk for stroke. (Stroke. 1994;25:1390-1392.)

Key Words • cerebral ischemia, transient • leukocytes • risk factors

During ischemic stroke, the extent of parenchymal tissue damage relates closely to the level of blood flow during the ischemic period and to the duration of ischemia.1 Because an increase of blood viscosity seems to play a central role in the impairment of cerebral blood flow, attention has been focused on the hemorheology of the cerebral circulation in stroke.2,3 Leukocytes have been studied mainly in regard to the functional and morphological properties that seem to qualify these cells for a central role in ischemic damage.4

Leukocytosis5 and altered leukocyte rheology6-7 have been described in the early phase of stroke, but the relevance of these findings in the development of ischemic damage remains controversial.8 In particular, it is not clear whether these hemorheologic changes can be regarded only as a reaction to the ischemic lesion in the brain or whether they may also play an initiating role in the etiology of cerebrovascular disease.

The current case-control study sought to verify whether an alteration of leukocyte aggregation, a factor that we have recently found increased in the first hours after ischemic stroke9,10 and which is supposed to increase blood viscosity, is also present in patients at risk for stroke. The evaluation was performed on patients that had recently suffered from atherothrombotic stroke or transient ischemic attack (TIA).

Subjects and Methods

The participants in the study were recruited from consecutive outpatients attending the Stroke Center of the Clinic of Neurology, "Tor Vergata" University of Rome during a 6-month period. Inclusion criteria were a history of ischemic stroke or TIA in the preceding 3- to 6-month period and the presence of a stenosis greater than 70% affecting the internal carotid artery or a stenosis greater than 50% of any major cerebral artery stem on the side of the symptomatic hemisphere detected by ultrasonic studies and/or by angiography. Exclusion criteria were an identified cardioembolic source and more than one episode of cerebral ischemic event.

Sixty-one patients entered the study. The acute event, according to the clinical history and to computed tomography, was classified as TIA in 21 patients and as stroke in 40 patients. The 21 women and 40 men had an average age of 66±6.1 years. Each patient was compared with a control subject of the same sex and of similar age (±5 years). No control subject had risk factors for cerebrovascular disease were evaluated in patients and in control subjects. Each patient with a given risk factor was matched with a control subject with the same risk factor. When more than one factor was present in the same patient, the following priority order was chosen in control subjects: hypertension, diabetes, smoking, elevated blood lipid levels, obesity, hyperuricemia, and alcoholism. Therefore, a perfect balance between patients and control subjects was present for hypertension (18 versus 18), whereas slight differences were present for diabetes, smoking, and elevated blood lipid levels (7 versus 6, 8 versus 10, and 6 versus 5, respectively). No other risk factor was observed in our population study.

Control subjects were recruited from consecutive subjects undergoing a cardiovascular assessment for the prevention of major atherosclerotic syndromes.

All the subjects of the study were without known hematologic disorders or inflammatory diseases, and no one took any drugs for at least 2 weeks before the study, except antidiabetic drugs and diuretics. Platelet antiaggregant agents, calcium entry blockers, and other drugs with a possible hemorheologic action were interrupted for 1 week before blood collection.

Informed consent was obtained from all subjects.

Leukocyte aggregation was evaluated by means of the leukergy test11 modified by us and described elsewhere.12 The evaluation was performed by a cytologist unaware of the clinical status of the subjects of the study. In all subjects a complete blood cell count was performed to avoid possible misinterpretation of leukocyte aggregation due to different factors.
conditions of blood concentration. The blood collection was performed at 9 AM after an overnight fast and smoking abstention.

The results are presented as mean±SD. Statistical evaluation was performed by using Student's t test to compare blood cell count and leukocyte aggregation values in patients and control subjects. The same test was used to compare stroke patients with TIA patients. To evaluate the possible interference of a different risk factor profile for vascular disease with levels of leukocyte aggregation and blood cell count, Student's t test was also used to compare subjects with and without risk factors. Kruskal-Wallis one-way ANOVA and Bonferroni's correction for multiple comparisons were used to evaluate the possible differential weight of a single stroke risk factor in altering leukocyte aggregation and blood cell count. For this purpose, patients and control subjects with risk factors were analyzed as a single group.

**Results**

There was no difference in blood cell count between control subjects and patients. Moreover, no difference was detected between TIA and stroke patients (Table 1).

The percentage of aggregated leukocytes was significantly higher in patients than in control subjects (3.8±3.4% versus 2.5±2.2%; P=.01), whereas no difference between patients with stroke and TIA was detected (3.9±3.7% versus 3.5±3.1%, respectively).

In the patient group, 31 subjects were without risk factors for stroke and 30 had risk factors (23 with a single factor and 7 with multiple factors). Values of leukocyte aggregation were not statistically different in these two subgroups (3.8±3.2% and 3.8±3.8%, respectively).

Similar to the patient group, 31 control subjects (mean age, 67±5.8 years) were without risk factors, whereas 30 (mean age, 64±8.9 years) had risk factors (23 with a single factor and 7 with multiple factors). A significant difference (P<.002) of the percentage of leukocyte aggregation was detected between the two subgroups. In fact, whereas the subjects without risk factors had values of leukocyte aggregation of 1.4±1.3%, those with risk factors had values comparable to those of patients, at 3.5±2.6%. No difference in the blood cell count was detected in patients and control subjects with and without vascular risk factors (Table 2).

Finally, the analysis performed in 60 subjects with vascular risk factors (30 patients and 30 control subjects) did not show differential weight of a single factor in altering leukocyte aggregation (range, 3.9±2.3% in smokers and 3.1±2.7% in subjects with elevated blood lipid levels) and blood cell count.

**Discussion**

Recent evidence suggests that leukocytes can exert a role in the pathogenesis of brain ischemic damage. Experimental and clinical studies showed an accumulation of these cells in low-flow regions in the first hours after ischemia.13 14 Central nervous system infiltration and neuronal cytotoxic injury have been postulated as modes of leukocyte potentiation of ischemia.15 In addition to their role in inflammatory response, the possibility of a direct mechanical obstruction of capillaries is suggested by studies on the morphological and physical characteristics of these cells and by clinical and experimental observations. The large volume, the high cytoplasmic stiffness, and the great coefficient of viscosity lead to an entry time into capillaries that is three orders of magnitude greater than for red blood cells.16 Moreover, clinical studies showed a decreased filterability and increased adhesive properties of granulocytes in ischemic stroke. Finally, in experimental models of cerebral ischemia, the reduction of leukocyte adhesiveness to endothelium provided a reduction of neurological deficit.17 18

Although the influx of leukocytes into injured ischemic tissue and the subsequent hemorheologic and biochemical reactions that could contribute to ischemic damage are believed to represent a response to the existing injury, the possibility that white cells may also play a role in the chain of events leading to the impairment of cerebral circulation and then to the induction of the ischemic event has been suggested. This hypothesis is also supported by the evidence that an elevated white blood cell count is a predictor of cerebral ischemia.19

<p>| Table 1. Blood Cell Count in Control Subjects and in Patients With Transient Ischemic Attack and Stroke |
|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>TIA Patients</th>
<th>Stroke Patients</th>
</tr>
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<tbody>
<tr>
<td>Hematocrit, %</td>
<td>43.3±5.4</td>
<td>42.2±4.1</td>
<td>44.7±3.4</td>
</tr>
<tr>
<td>WBC, U×10^9/mm³</td>
<td>6.2±2.1</td>
<td>6.4±1.9</td>
<td>6.7±3.2</td>
</tr>
<tr>
<td>Platelet, U×10^9/mm³</td>
<td>252.1±58</td>
<td>259.3±83.7</td>
<td>256.2±65.1</td>
</tr>
</tbody>
</table>

TIA indicates transient ischemic attack; WBC, white blood cell count. Values are mean±SD.

<p>| Table 2. Blood Cell Count in Control Subjects and in Patients With and Without Vascular Risk Factors |
|---------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| Control Subjects                                                                                     | Patients                                                                                         |
|                                                                                                    |                                                                                                  |</p>
<table>
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<tr>
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<th>With Risk Factors</th>
<th>Without Risk Factors</th>
<th>With Risk Factors</th>
<th>Without Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td>44.6±5.1</td>
<td>42.1±5</td>
<td>41.8±6</td>
<td>43.5±3.9</td>
</tr>
<tr>
<td>WBC, U×10^9/mm³</td>
<td>6.2±1.5</td>
<td>6.3±1.6</td>
<td>6.8±2.2</td>
<td>8.3±2</td>
</tr>
<tr>
<td>Platelet, U×10^9/mm³</td>
<td>244.2±50.5</td>
<td>259.8±46</td>
<td>261.6±90.9</td>
<td>253±68.7</td>
</tr>
</tbody>
</table>

WBC indicates white blood cell count. Values are mean±SD.
In the present study we evaluated leukocyte aggregation, a particular aspect of leukocyte rheology potentially able to impair cerebral microcirculation, in patients with a previous cerebral ischemic event and in control subjects with and without vascular risk factors. The percentage of aggregated leukocytes found in patients and in control subjects with risk factors was increased compared with control subjects without risk factors and similar to that previously detected in patients in the first hours after a TIA or minor stroke. These findings do not allow any speculation on the possible pathogenetic significance of changes of leukocyte rheology in cerebrovascular disease. However, the presence of a persistently altered status of leukocyte aggregation in patients with a recent cerebral ischemic event as well as in subjects with vascular risk factors suggests that this aspect of leukocyte rheology cannot be considered a simple inflammatory reaction occurring during the development of ischemic damage. Further investigations and particularly follow-up studies should be performed to evaluate whether the alteration of leukocyte aggregation is associated with an increased risk for the occurrence of cerebral ischemic events.

References
Leukocyte aggregation in patients with a previous cerebral ischemic event.
M Silvestrini, A Pietroiusti, A Magrini, M Matteis, S Carta, G Bernardi and A Galante

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