Hemorheological Factors in Cerebral Ischemia

Mark Fisher, MD, and Herbert J. Meiselman, ScD

We investigated 100 consecutive cerebral ischemia patients for hemorheological alterations. We measured whole and adjusted blood viscosity at 75 and 1,500 sec⁻¹, plasma viscosity, red blood cell aggregation by the zeta sedimentation ratio, and red blood cell deformability using the centrifugal deformability technique. Patients were studied within 72 hours of the acute ischemic event, and 66 were available for follow-up evaluation approximately 2 months later. Two age- and sex-matched control groups were evaluated: 20 nonvascular neurological inpatients (patient controls) and 45 normal volunteers (normal controls). Compared with normal controls, we found significant acute increases in whole blood viscosity (1,500 sec⁻¹), plasma viscosity, fibrinogen concentration, and zeta sedimentation ratio; the latter two variables were also increased at follow-up. Fibrinogen concentration was significantly associated with zeta sedimentation ratio and plasma viscosity and was increased for patient controls. There was a trend toward normalization of acute abnormalities over the 2-month follow-up period, and patients with more severe strokes tended to have more extensive hemorheological abnormalities. Among patients with severe stroke, fibrinogen concentration was significantly associated with the platelet activation peptide β-thromboglobulin acutely (r=0.63, p<0.005). We conclude that hemorheological abnormalities in cerebral ischemia are largely nonspecific findings, with the likely exception of patients with severe stroke.

From the Departments of Neurology (M.F.) and Physiology and Biophysics (H.J.M.), University of Southern California School of Medicine, Los Angeles, Calif.

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Address for reprints: Mark Fisher, MD, Department of Neurology, University of Southern California School of Medicine, 2025 Zonal Avenue, Los Angeles, CA 90033.

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Patients were classified as having TIA or stroke based on the duration of ischemic event. Clinical, CT, and, when available, arteriographic information was used to classify patients with regard to etiology. Four etiologic classifications were established: 1) large-vessel occlusive disease, consisting of symptoms of internal carotid, anterior cerebral, middle cerebral, posterior cerebral, or vertebrobasilar insufficiency; CT scans and, when available, arteriography, provided confirmatory details; 2) cardiogenic processes, consisting of the presence of atrial fibrillation or valvular disease, electrocardiographic evidence of (nonrecent) myocardial infarction with echocardiographic demonstration of an embolic source, or (nonrecent) myocardial infarction in a patient with large-vessel symptoms and normal cerebral arteriography; 3) lacunar disease, consisting of clinical evidence for any of the lacunar syndromes, that is, pure motor hemiparesis, pure sensory stroke, ataxic hemiparesis, and dysarthria–clumsy hand syndrome, with CT scan either normal or demonstrating small, deep infarcts, and a history of hypertension; and 4) other–undefined, consisting of patients who did not clearly fit into any of the other three standard etiologic syndromes.

All patients were personally examined and were given a neurological function score based on a standardized examination as previously described. Scoring was as follows: level of consciousness, 12 points maximum; speech, language, facial movements, light touch, superficial pain, joint position, stereognosis, extinction, finger–nose, and heel–knee–shin examination, 2 points maximum for each; conjugate eye movements, 3 points maximum; upper extremity strength and lower extremity strength, 5 points maximum for each. A maximum score of 50 points is taken as a normal examination. Thirty points or less was considered a severe deficit, and 31–49 points a mild deficit.

Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) vacuum tubes (1.5 mg/ml blood), and rheological measurements were completed within 5 hours after venipuncture. No samples were drawn within 24 hours after a radiographic contrast procedure. Hematocrit was determined using the microhematocrit technique, and fibrinogen levels were determined by the turbimetric measurement of the formation of fibrin polymer (Du Pont ACA, Wilmington, Del.). White blood count (WBC) and differential were determined by an automated counter (Coulter S+IV, Coulter Electronics, Hialeah, Fla.).

Whole-blood viscosity was measured with a Wells-Brookfield Micro Cone-Plate Viscometer (Brookfield Engineering Laboratories, Stoughton, Mass.), operating at 25°C, over a shear rate range of 75-1,500 sec⁻¹. Values at 75 and 1,500 sec⁻¹ were used for data analysis. In addition, viscosity was measured for RBC-plasma suspensions, adjusted to 40% hematocrit, over the same shear rate range; again, values of this adjusted blood viscosity at 75 and 1,500 sec⁻¹ were used for data analysis. Plasma viscosity was measured at 750 and 1,500 sec⁻¹ using the same cone-plate viscometer, with values averaged for data analysis. All viscosity data are reported as centipoise (cp), where 1 cp = 1 mPa/sec; the viscosity of water at 25°C is 0.895 cp.

Red blood cell aggregation was quantified by the zeta sedimentation ratio, which defines the degree of RBC aggregation after a 3-minute period of low-gravity (7–8g) dispersion and compaction. Cell suspensions are placed in a 75x2-millimeter-i.d. glass tube and rotated for four 45-second periods in a near-vertical position in a Zetafuge (Coulter). The RBC-suspending medium after the 3-minute centrifugation determines the "zetacrit" of the suspension, and (zetacrit x microhematocrit) x 100 = zeta sedimentation ratio. Increases in zeta sedimentation ratio indicate increasing RBC aggregation.

Erythrocyte deformability was determined using a high-speed centrifugation system. The basic portion of this system consists of a horizontally positioned high-speed microfuge (model 152, Beckman Instruments, Inc, Fullerton, Calif.). Microfuge tubes of 0.55-ml capacity are used with a Teflon insert designed to fit inside the tube and extend to 1 cm of the bottom of the tube. Three fluids are placed, in succession, in the microfuge tube before centrifugation: 75 μl 2% glutaraldehyde, 100 μl isotonic buffer, followed by placement of the Teflon insert, and 3–4 μl of a dilute RBC-isotonic buffer suspension. The microfuge tube is spun at 16,000 rpm for 5 seconds; the cells are deformed during their passage through the isotonic buffer and are fixed in their deformed state by the glutaraldehyde. The mean length of 30 RBC (in microns) is taken as an index of RBC deformability, with increasing length indicating increasing deformability.

Plasma for β-thromboglobulin and platelet factor 4 was collected using a modification of the method of Files et al. A precooled solution of 1 ml acid-citrate-dextrose (ACD, NIH formula A), 80 μl aspin (180 mg ASA/ml ethanol), and 10 μl PGE1 (100 μg PGE1/ml ethanol) was prepared just before venipuncture. The solution was drawn into a syringe, which was then placed on ice. Using a 21-gauge "butterfly," 4 ml blood was drawn directly into the syringe without use of a tourniquet. All venipunctures were performed by one individual. Plasma samples were analyzed only if venipuncture resulted in prompt flow into the syringe; without stasis. The syringe was then inverted 10 times and placed on ice for not more than 30 minutes, and the blood was centrifuged at 10,000g for 30 minutes. Plasma aliquots of approximately 100 μl were then stored frozen before measurement of β-thromboglobulin and platelet factor 4 levels. β-thromboglobulin was measured by radioimmunoassay (Amersham Co., Chicago, Ill.). To confirm the absence of in vitro platelet release, platelet factor 4 levels were also determined by enzyme immunoassay (American Bio-Products Company, Parsippany, N.J.). All β-thromboglobulin levels for patients and patient controls...
Table 1. Rheological Data for All Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
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<tr>
<td></td>
<td>Acute</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Number</td>
<td>100</td>
<td>66</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.9±11.7</td>
<td>52.0±11.4</td>
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<tr>
<td>Hematocrit</td>
<td>42.0±5.0</td>
<td>40.3±4.9</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>388±89t</td>
<td>374±118t</td>
</tr>
<tr>
<td>Whole-blood viscosity, 75 sec(^{-1}) (cP)</td>
<td>9.15±1.86</td>
<td>8.51±1.80</td>
</tr>
<tr>
<td>Whole-blood viscosity, 1,500 sec(^{-1}) (cP)</td>
<td>4.98±0.65†</td>
<td>4.82±0.62</td>
</tr>
<tr>
<td>Plasma viscosity (cP)</td>
<td>1.84±0.16†</td>
<td>1.82±0.15</td>
</tr>
<tr>
<td>Adjusted blood viscosity, 75 sec(^{-1}) (cP)</td>
<td>9.72±1.82</td>
<td>9.42±2.13</td>
</tr>
<tr>
<td>Adjusted blood viscosity, 1,500 sec(^{-1}) (cP)</td>
<td>5.05±0.40</td>
<td>5.06±0.51</td>
</tr>
<tr>
<td>Zeta sedimentation ratio</td>
<td>59.2±6.3§</td>
<td>57.7±5.9§</td>
</tr>
<tr>
<td>RBC deformability (µm)</td>
<td>11.2±0.4</td>
<td>11.1±1.3</td>
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</table>

*Fibrinogen data for 96 acute patients.
†p<0.025, ‡p<0.0025, §p<0.05 increased compared with normal controls by unpaired Student's t test.

Elevated beyond 2 SDs above the mean of normal controls (12.2±6.5 ng/ml), and in which simultaneous platelet factor 4 levels were increased more than 3 SDs above the mean of normal controls (1.4±1.6 ng/ml), were considered to represent in vitro release of β-thromboglobulin and eliminated from data analysis.

Two control groups were used for data analysis. Patient controls consisted of a group of neurological inpatients hospitalized for nonvascular problems. Normal controls consisted of a group of healthy volunteers. Statistical analysis was performed using unpaired Student's t tests and simple linear correlation. Bonferroni corrections were made when appropriate.

Results

As shown in Table 1, the acute patients demonstrated significant elevations compared with normal controls for fibrinogen concentration, whole blood viscosity at 1,500 sec\(^{-1}\), plasma viscosity, and zeta sedimentation ratio. At follow-up, significant elevations persisted for both fibrinogen concentration and zeta sedimentation ratio. Compared with normal controls, patient controls had significant elevations for fibrinogen only.

Subset analyses were performed on those variables increased in acute patients versus normal controls (Table 1), with these etiologic subset results shown in Table 2. Salient aspects of Table 2 include the following: 1) fibrinogen concentration was elevated, both acutely and at follow-up, in all three etiologic subsets; 2) whole-blood viscosity (1,500 sec\(^{-1}\)) was increased acutely only for the cardiogenic and lacunar patients; 3) plasma viscosity was above normal control levels for the acute cardiogenic and lacunar patients, but was elevated only for the large-vessel subset at follow-up; and 4) zeta sedimentation ratio demonstrated increases only for the acute large-vessel and cardiogenic patients.

Subset evaluations based on severity were also carried out for both fibrinogen (Figure 1) and for total WBC count as well as for WBC differential results (Table 3). Dealing first with fibrinogen, note that elevations were found only in patients with stroke and that it was persistently increased for both stroke groups; further, it is notable that the absolute levels of fibrinogen were greater for the severe versus mild stroke patients (i.e., 10% larger acutely, 17% at follow-up). The WBC data (Table 3), obtained for a somewhat smaller number of patients and patient
controls, indicate significantly increased total WBC counts for all acute patients (20% above patient controls) and that this increase was primarily due to the severe stroke subset (40% elevation). The elevated WBC level was predominately granulocytic, although there was a significant increase of 29% for lymphocytes of the acute mild stroke group.

Linear regression analyses indicated highly significant associations (p < 0.005) both acutely (100 patients) and at follow-up (66 patients) for the following: hematocrit and whole-blood viscosity at 1,500 sec⁻¹ (r = 0.81 acutely and 0.79 at follow-up), fibrinogen concentration and plasma viscosity (r = 0.49 and 0.64), and fibrinogen concentration and zeta sedimentation ratio (r = 0.56 and 0.61). Plasma viscosity was also significantly (p < 0.0005) associated with adjusted blood viscosity at 75 sec⁻¹ (r = 0.41 and 0.50), adjusted blood viscosity at 1,500 sec⁻¹ (r = 0.59 and 0.43), and zeta sedimentation ratio (r = 0.63 and 0.68).

Plasma β-thromboglobulin values in this cohort have been described previously, with both acute and follow-up patients demonstrating significant increases compared with controls. To investigate a link between coagulation and hemorheological variables, we evaluated associations between β-thromboglobulin and fibrinogen. There was a significant association between fibrinogen and β-thromboglobulin for all stroke patients acutely (r = 0.33, n = 57, p < 0.02), but not for stroke patients at follow-up (r = 0.13, n = 41) or for TIA patients studied acutely or at follow-up (r = 0.01, n = 25 and r = 0.32, n = 16, respectively). Among stroke patients studied acutely, the β-thromboglobulin-fibrinogen association was significant for those with severe stroke (r = 0.63, n = 16, p < 0.005; Figure 2), but not for those with mild stroke (r = 0.14, n = 41).

**Discussion**

We have demonstrated significant hemorheological abnormalities in patients with acute cerebral ischemia: increases in whole-blood viscosity at high shear rate (1,500 sec⁻¹), plasma viscosity, zeta sedimentation ratio (i.e., RBC aggregation), and fibrinogen concentration. However, neither hematocrit, blood viscosity at 40% hematocrit, nor RBC defor-

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**Table 3. White Blood Cell Count**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Granulocytes</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
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<tbody>
<tr>
<td>Transient ischemic attack (acute)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 23</td>
<td>7.05 ± 1.47</td>
<td>4.60 ± 1.36</td>
<td>2.02 ± 0.55</td>
<td>0.44 ± 0.22</td>
</tr>
<tr>
<td>Mild stroke (acute)</td>
<td>7.63 ± 2.07</td>
<td>5.14 ± 1.76</td>
<td>2.17 ± 0.82**</td>
<td>0.39 ± 0.23</td>
</tr>
<tr>
<td>n = 38</td>
<td></td>
<td></td>
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<tr>
<td>Severe stroke (acute)</td>
<td>9.22 ± 3.09**</td>
<td>7.00 ± 3.16*</td>
<td>1.73 ± 0.61</td>
<td>0.47 ± 0.31</td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
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<tr>
<td>All patients (acute)</td>
<td>7.85 ± 2.34*</td>
<td>5.44 ± 2.28</td>
<td>2.02 ± 0.72</td>
<td>0.42 ± 0.25</td>
</tr>
<tr>
<td>n = 51</td>
<td></td>
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<tr>
<td>All patients (follow-up)</td>
<td>7.65 ± 2.52</td>
<td>5.26 ± 2.24</td>
<td>2.01 ± 0.80</td>
<td>0.34 ± 0.21</td>
</tr>
<tr>
<td>n = 55</td>
<td></td>
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<tr>
<td>Patient controls</td>
<td>6.54 ± 2.29</td>
<td>4.52 ± 2.61</td>
<td>1.69 ± 0.61</td>
<td>0.33 ± 0.24</td>
</tr>
<tr>
<td>n = 14</td>
<td></td>
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Units are per mm³ × 10³.

*p < 0.05, **p < 0.005 increased compared with patient controls by unpaired Student's t test.

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**Figure 1. Plasma fibrinogen levels in stroke patients and controls. TIA, transient ischemic attack. *p < 0.025, **p < 0.0025 compared with normal controls.**
morbidity, as measured by centrifugal deformation, was altered. There was a trend toward normalization of high shear rate whole-blood viscosity and plasma viscosity at follow-up 2 months later, although zeta sedimentation ratio and fibrinogen concentration still remained significantly elevated. The three major etiologic subsets demonstrated similar trends, whereas comparisons by deficit (TIA versus mild stroke versus severe stroke) showed a trend toward more substantial abnormalities in the more severely affected patients. Fibrinogen levels were significantly associated with levels of the platelet activation protein β-thromboglobulin for acute stroke patients. In a somewhat smaller group of patients, WBC were increased acutely, most notably among patients with severe strokes, compatible with prior reports.\(^{15,16}\) Increased WBC, along with impaired WBC filterability,\(^{15,16}\) may contribute to perfusion impairment in the acute stroke period.

The finding of significant increases in hemorheology variables compared with normal controls is perhaps less revealing than the analysis of the patient control group. The latter patients, representing a group of nonvascular neurology inpatients, had elevations of fibrinogen concentration roughly similar to that of both acute and follow-up cerebral ischemia patients. The increased fibrinogen in the patient control group, along with the high correlation between fibrinogen and both zeta sedimentation ratio and plasma viscosity, suggest that the acute increases of the latter two variables are largely nonspecific. The remaining increased variable, high-shear whole-blood viscosity, is best explained by the slight increase in hematocrit in acute ischemic patients; high shear blood viscosity, with hematocrit adjusted to 40%, was not increased.

The major exception to the apparent nonspecificity of these findings is the subset of patients with severe stroke. This group of patients had markedly increased acute levels of fibrinogen (mean±SD=427±98 mg/dl, range 293–636), with no evidence of normalization at follow-up (Figure 1), raising the possibility that elevated fibrinogen may have preceded the stroke. This finding is of interest given the multiplicity of observations relating fibrinogen and stroke, including the following: increased fibrinogen is a stroke risk factor, at least in males;\(^{17,18}\) increased fibrinogen is associated with progression of carotid atherosclerosis;\(^{19}\) and there is an inverse relation between fibrinogen and cerebral blood flow and, in elderly individuals, middle cerebral artery blood velocity.\(^{20}\) An additional potential link between fibrinogen and cerebral ischemia is the finding in the current study of a significant association between levels of fibrinogen and the platelet activation protein β-thromboglobulin in patients with acute stroke. Fibrinogen is a major link between aggregating platelets via the glycoprotein IIb–IIIa complex;\(^{21}\) aggregation is a component of the platelet activation response. The fibrinogen–β-thromboglobulin association is therefore not surprising and suggests a possible link between stroke risk, fibrinogen levels, and platelet activation. Moreover, the fibrinogen–β-thromboglobulin association was not seen for patients with TIA or mild stroke, thus lending additional support for the concept that hemorheological factors play a distinct role for patients with severe stroke.

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References


**KEY WORDS** • blood coagulation • blood platelets • cerebral ischemia • fibrinogen
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