Blood Cell Rheology in Acute Cerebral Infarction

Michele Mercuri, MD, Giovanni Ciuffetti, MD, Martin Robinson, MB, BS, and James Toole, MD

Recently it has been hypothesized that leukocyte rheology could be a relevant variable of the microcirculation during cerebral ischemia. However, relatively few studies have been carried out on the rheologic behavior of leukocytes in vascular diseases. This study aimed at quantifying the filterability through Nuclepore filters (mean pore diameter 5 μm) of both leukocyte subpopulations and red blood cells in patients with acute stroke compared with age-matched healthy controls. Leukocytes were separated by density into polymorphonuclear and mononuclear cells. Filterability of the red blood cells and polymorphonuclear and mononuclear subpopulations in buffer was measured using a constant-flow and low-positive pressure system. We used one-way analysis of variance, signed rank sum, and simple and multiple regression tests for statistical analysis. Twenty consecutive male patients with acute ischemic infarction were compared with 20 age-matched healthy subjects. Mononuclear cell filterability was impaired in acute stroke (7.26±2.00) compared with the controls (5.55±1.23) (p<0.01). Polymorphonuclear cell filterability was less, but still significantly (p<0.05), impaired in acute infarction (5.75±0.87 vs. 4.19±0.43). The results show that leukocyte and, especially, mononuclear cell filterability is impaired in acute infarction, while no differences exist in red blood cell filterability. (Stroke 1989;20:959–962)

Attention has recently been directed to the putative rheologic role of blood cells, particularly leukocytes (WBC), in ischemic arterial disease. Studies on both animals and humans have underlined that occlusion of capillaries by WBC is a possible contributing factor to the “no-reflow” phenomenon. A tentative explanation for this is that WBC are more rigid, larger, and more adhesive than red blood cells (RBC) and by adherence to vascular endothelium could perturb normal flow dynamics in the microcirculation. Furthermore, under ischemic conditions leukocytes may become activated and release leukotrienes and prostaglandins, which may further impair the microcirculation.

The objectives of this study were to investigate whether RBC and WBC subpopulations (polymorphonuclear [PNL] and mononuclear [MNL] leukocytes) had a different filterability; whether individual WBC subpopulations were affected differently; whether correlations existed between blood cell counts and blood cell filterability; and whether correlations existed between Canadian Neurological Scale scores and blood cell subpopulation filterabilities during the acute phase of cerebral infarction (i.e., within 24 hours of onset).

Subjects and Methods

This study was performed between July and December 1987 at the Internal Medicine Department II, University of Perugia (Italy). Twenty consecutive male patients, admitted within 24 hours of onset of acute ischemic infarction (mean 14.5±2.8 hours) were compared with 20 age- and sex-matched healthy control subjects. Patients were clinically assessed and graded according to the Canadian Neurological Scale. After informed consent, admission to the study occurred if the immediate cranial computerized tomography showed no evidence of intracranial hemorrhage. Patients with a Glasgow Coma Scale score of less than 8 were excluded. None of the patients were taking anticoagulant or antiplatelet medications on admission to the study. This was assessed by direct questioning of the patient, checking medications, and reviewing the medical records.
Objections could be raised concerning our choice to perform only one measurement instead of several such evaluations after the initial acute infarction. Our reason for this was that after admission to the hospital, treatment of each patient was individualized. Patients received different fluid regimes and medications. The effect of these interventions on blood cell filterability is unknown. Therefore we performed our experiment before any treatment was started. Treatment could be a confounding variable in later examinations.

Controls were selected from a healthy group of people. None had any history of cardiovascular disease and were free of the major risk factors for atherosclerosis (i.e., hypertension, smoking, diabetes, and hyperlipidemia). Furthermore, none of the controls suffered from any other major illness or had taken any medications within the previous 20 days. This information was ascertained by direct patient questioning and routine physical examination.

Flowing blood was sampled from an arm vein and anticoagulated with dry dipotassium edetate (EDTA 1.5 mg/ml). RBC and WBC counts were determined by an automated hematology analyzer. The separation and filtration of the cells were performed as follows.

PNL and MNL were separated using Ficoll-Hypaque density gradient (1.114 g/ml and 1.022 g/ml) after centrifugation. Cells were washed and resuspended in phosphate-buffered saline (PBS) (pH 7.4, 290 mosm/kg), containing 0.5% bovine serum albumin at a concentration of 1,000,000 cells/ml. RBC contamination of the white blood cell suspension was not detectable. RBCs were separated after a 10-minute centrifuge at 3,000g. The plasma buffy coat and upper 10% of packed RBCs were aspirated and discarded. RBCs from the middle part of the RBC column were suspended in PBS at a 10% hematocrit. Each sample of RBC suspension was diluted 1:10 in 2% glacial acetic acid to which a few grains of crystal violet stain had been added. WBC contamination was then checked in a Neubauer counting chamber using a light microscope. WBC contamination in the RBC suspension was less than 0.1 x 10^9/l. Platelet contamination was not detectable.

Each suspension sample was filtered using a constant-flow syringe pump at 1.5 ml/min for 6 minutes. The pressure rise was monitored by a pressure transducer linked to an amplifier while chart recorder filterability was expressed as the final filtration pressure generated by the cell suspension relative to the buffer, previously filtered through the same filter. A single batch (No. 5414C32) of Nuclepore (Pleasanton, California) membranes with nominal pore diameter 5 μm, filter diameter 13 mm, and effective filtration area 0.78 cm^2 was used for filtration throughout the study. Filtration was performed within 3 hours of venipuncture. The filtration tests were carried out at a standard concentration of cells to avoid effects due to changes in absolute cell counts.

Reliability and quality of the method was confirmed by five successive filtrations of cell suspensions giving the mean coefficient of variance. After 6 minutes of filtration, the values were 4.6% for MNL, 4.4% for PNL, and 2.3% for RBC.

Results

Statistical analysis was performed using parametric (one-way analysis of variance [ANOVA]), nonparametric (Wilcoxon signed rank sum test), simple, and multiple regression analysis. As blood cell counts are approximately normally distributed, ANOVA was used to analyze that particular data. Since the pattern of distribution of blood cell filterability is unknown, nonparametric tests were used to analyze that data set. Simple and multiple regression tests were finally used to describe relations between blood cell filterabilities and neurologic scores.

Table 1 displays our findings. ANOVA showed significant differences in WBC counts between the stroke and control groups (p < 0.001).

Nonparametric tests showed the WBC filterability (pressure ratio of cell suspension to buffer after 6-minute filtration) was impaired in the acute stroke group as compared with controls (7.26±2.00 vs. 5.55±1.23, p < 0.01, for MNL, and 5.75±0.87 vs. 4.19±0.63, p < 0.05, for PNL).

MNL filterabilities showed a large standard deviation compared with that of the PNL. Since the means of filterabilities for PNL and MNL are significantly different, the coefficient of variation (CV), which expresses the standard deviation as a percentage of the mean, could be more appropriate for comparing these results. CVs were 0.15 in both case and control PNL filterabilities and 0.27 and 0.22, respectively, in case and control MNL filterabilities.

RBC filterability was not significantly different between groups. No relation was found between the blood cell counts, initial Canadian Neurological Scale, and subpopulation filterability values.

### Table 1. Rheological Characteristics of Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Acute cerebral infarction (n=20)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>Mean 55 SD 7</td>
<td>Mean 54 SD 5</td>
</tr>
<tr>
<td>RBC count (10^9/l)</td>
<td>4.60 ± 0.30</td>
<td>4.60 ± 3.90</td>
</tr>
<tr>
<td>WBC count (10^9/l)</td>
<td>8.18 ± 1.64</td>
<td>6.03 ± 1.28</td>
</tr>
<tr>
<td>RBC filterability*</td>
<td>3.03 ± 0.83</td>
<td>2.99 ± 0.73</td>
</tr>
<tr>
<td>PNL filterability*</td>
<td>5.75 ± 0.87</td>
<td>4.19 ± 0.63</td>
</tr>
<tr>
<td>MNL filterability*</td>
<td>7.26 ± 2.00</td>
<td>5.55 ± 1.23</td>
</tr>
</tbody>
</table>

RBC, red blood cell; WBC, leukocyte; PLN, polymorphonuclear cell; MNL, mononuclear cell.

*Pressure ratio of cell suspension to buffer after 6 minutes filtration.
Discussion

WBCs have been recognized as playing a role in the pathogenesis of both atherosclerosis and ischemia. Studies have outlined the role of blood cells in the regulation of microvascular flow. Recently, it has been suggested that alteration of whole blood filterability in vascular diseases is related more to WBC than to the previously held principle of RBC rigidification. Our study was designed to evaluate the rheology of blood cell subpopulations (RBC, PNL, MNL) during the acute phase of ischemic stroke (less than 24 hours) introducing two new conditions: observation during the acute phase of ischemia and study of the individual subpopulations. The filtration method used (a technique derived by Lennie et al) is able to investigate RBC and WBC subpopulations under similar conditions, while it is impossible to study platelet effects and biohumoral influences (i.e., fibrinogen) because of their loss during the separation procedures. Although these procedures may be used for determining the role of blood cells in cerebral ischemia, it does not enable one to make conclusions about possible mechanisms leading to the altered filterability.

Our results showed that there were no differences in RBC filterability between cases and controls. On the contrary, WBC filterability, especially that of the MNL subgroup, is significantly different from that of an age- and sex-matched control group. MNL filterability showed a large coefficient of variation compared with that of PNL, but this does not reduce the significance of the results. In fact, an explanation is the presence of two significantly different filtering subpopulations in MNL, lymphocyte and monocyte. This hypothesis is supported by our current studies of the individual granulocyte, lymphocyte, and monocyte filterabilities. It could be questioned that impairment of WBC filterability is due to underlying atherosclerosis rather than to the acute infarction per se. Previous studies in patients with coronary and peripheral artery disease have shown no differences between cases and matched controls at rest, while WBC became less filterable when exercise-induced ischemia occurred. This suggests that the ischemia rather than the atherosclerosis contributes to impaired filterability. The mechanisms leading to the altered filterability of WBCs and how this may potentiate cerebral ischemia is not understood. Under normal conditions, WBCs’ delay in entering capillaries is 1,000 times greater than that of the RBCs; thereafter WBCs progress through the capillaries with a much lower velocity than do RBCs. These factors may lead to a temporary, physiologic occlusion of the capillaries. When perfusion pressure is reduced, as occurs in ischemic cerebral infarction, WBCs may not be able to pass through the capillaries and may adhere to endothelium. Furthermore, WBC activation may worsen local ischemia by release of vasoactive substances, while mechanical obstruction of capillaries may impair oxygen delivery by the RBCs and may potentially increase the size of the infarct. WBCs are thought to be a potential factor responsible for the "no-reflow" phenomenon. This was first described in 1968 by Ames et al and currently is gaining much interest because it has been hypothesized that leukocyte activation after reperfusion of infarcted myocardium may lead to an extension of the infarcted area.

In agreement with other studies conducted at different time intervals after cerebral infarction, our findings suggest that leukocyte rheology could be an additional relevant variable of the microcirculation during acute cerebral ischemia. Studies in acute cerebral ischemia using rheologic modifying agents (e.g., pentoxifylline and hemodilution) targeted at the RBC have failed to show beneficial effects. This may be related to the understimation of the role of WBCs in the cerebral microcirculation during acute cerebral ischemia. Our results suggest that one must pay attention not only to RBC but also to WBC alterations during ischemia to more fully understand the pathophysiology of microvascular flow and to devise interventions for its correction.

Acknowledgment

The authors wish to express their gratitude to Ms. Karla Essick for her help in preparing the manuscript.

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Key Words • cerebral infarction • leukocytes • rheology
Blood cell rheology in acute cerebral infarction.
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Stroke. 1989;20:959-962
doi: 10.1161/01.STR.20.7.959

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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