Altered Leukocyte Rheology in Patients With Chronic Cerebrovascular Disease

I. Vermes, MD, PhD, and F. Strik, MD, PhD

Erythrocyte and leukocyte suspensions were prepared from 28 patients with chronic cerebrovascular disease and 30 age- and concomitant disease-matched control subjects. Cell filterability was studied with the new St. George’s filtrometer, which can discriminate between initial filtration rate as an erythrocyte parameter and filter clogging as a leukocyte parameter. Compared with control subjects, a significant increase of filter clogging was found in the patients, suggesting decreased deformability or increased adhesiveness of leukocytes or both in chronic cerebrovascular disease. (Stroke 1988;19:631–633)

It is generally accepted that hemorheologic alterations are present in about 50% of patients with cerebrovascular disease (CVD). In addition to vascular components, increased blood viscosity plays an important role in the impairment of cerebral blood flow in these patients. Several factors, such as changes of the qualitative and quantitative behavior of erythrocytes and platelets as well as changes in plasma composition, have been identified as being responsible for the deterioration of blood fluidity. Leukocytes are also known to influence blood fluidity, but due to methodological difficulties less attention has been paid to the rheologic properties of leukocytes in CVD. Recently, a new blood filtration technique was introduced that enables investigations of the mechanical properties of leukocytes. By using this technique Ernst et al demonstrated very recently a reduced leukocyte filterability in blood suspensions with standardized leukocyte and erythrocyte counts of patients with acute stroke. We investigated the filterability of isolated erythrocytes and leukocytes in patients with chronic CVD.

Subjects and Methods

Our study evaluated 28 patients with CVD and 30 control subjects without any evidence of CVD. The 12 women and 16 men with CVD had an average age of 66 ± 9 years, and 2 patients had a history of myocardial infarction, 8 of hypertension, and 2 of diabetes mellitus. In all patients at least 6 months had elapsed since their last cerebrovascular events. The last attack was classified according to the clinical history and computed tomography in 9 patients as transient ischemic attack (TIA) and in 19 patients as cerebrovascular accident (CVA). The control group consisted of 30 subjects matched for age (61 ± 11 years) and for concomitant diseases (1 patient had suffered a myocardial infarction, 6 had hypertension, and 4 had diabetes mellitus). There were no significant differences between patients and control subjects in terms of immobility, presence of infections, blood pressure, and hematologic indexes.

Venous blood was collected with minimal occlusion, and ethylenediaminetetraacetic acid was used as an anticoagulant. Hematocrit and hematologic indexes were determined by an automatic counter (Coulter Electronics Ltd., Luton, U.K.). Pathological hematologic indexes or an abnormal leukocyte count were exclusion criteria for this study. Erythrocyte and leukocyte suspensions were prepared according to published methods. Leukocyte contamination of the erythrocyte suspension was <0.1 x 10^9/L, and platelet contamination was not detectable. A hematocrit of 10% was used for the filtration measurements. Erythrocyte contamination of the leukocyte suspension was not detectable, and 1 x 10^9 cells/L was used for the filtration measurements. The differential leukocyte count was controlled after isolation, and only cell suspensions with a lymphocyte percentage of between 30% and 40% were included. The percent granulocytes in the final suspensions were 64 ± 4% in the CVD patients and 65 ± 4% in the control group.

The cell suspensions were filtered with a St. George’s filtrometer (Carri-Med Ltd., Dorking, U.K.) at 25°C, using polycarbonate membrane filters (Nuclepore, Pleasanton, California) batch no. 5404C48, nominal pore diameter 5 μm, filter diameter 13 mm, effective filtration area 0.78 cm², with a driving pressure of ~4 cm water. Phosphate-buffered saline (0.01 M, pH 7.4, osmolality 295 mosm/kg) was prefilted through a filter with a 1-μm-diameter pore to eliminate contaminating particles. The initial filtration rate of the cell suspensions was quantified by extrapolating the progressively decreasing filtration rate to time 0. Erythrocyte filterability was expressed as erythrocyte transit time. Filter clogging was determined from the decrease of filter conductance during the first 250 μl filtration, and the concentration of particles initially plugging the pores of the filter was used to express the leukocyte filterability. The coefficient of variation for erythrocyte transit time and for clogging particles based on 10 tests on a single suspension was <5%. All samples were measured in triplicate. Results are presented as mean ± SD, and

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occurrence of CVD. The whole blood viscosity as fluidity is present in the majority of patients with CVD, suggesting the contribution of hemorheologic alterations as accelerating factors in the development and occurrence of CVD [1][3][5]. The whole blood viscosity as an index of blood fluidity is a complex phenomenon including plasma, erythrocyte, platelet, and leukocyte components [1][2]. Impaired erythrocyte deformability, measured by the cell filtration technique, is believed to play a leading cause of the microcirculatory disturbances present in patients with CVD [1][4][6]. However, more recent studies have shown that blood filtration reflects not only erythrocyte deformability but also retention of leukocytes in the filter [9][10][11]. Early filtration methods could not differentiate between these two components influencing blood filtration. The recently described St. George's filtrometer is capable of differentiating between these two components by measuring both the initial filtration rate and erythrocyte transit time when filtering erythrocyte suspensions (Table 1). However, the number of clogging particles during erythrocyte filtration was significantly higher in CVD patients than in control subjects. These differences were more pronounced when filtering leukocyte suspensions (Table 1). In CVD patients significantly more clogging particles of leukocyte suspensions were found than in control subjects, without alteration of the initial filtration rates (Table 1).

**Results**

There were no significant differences between CVD patients and control subjects with respect to the initial filtration rate and erythrocyte transit time when filtering erythrocyte suspensions (Table 1). However, the number of clogging particles during erythrocyte filtration was significantly higher in CVD patients than in control subjects. These differences were more pronounced when filtering leukocyte suspensions (Table 1). In CVD patients significantly more clogging particles of leukocyte suspensions were found than in control subjects, without alteration of the initial filtration rates (Table 1).

**Discussion**

In the literature there is no doubt that decreased blood fluidity is present in the majority of patients with CVD, suggesting the contribution of hemorheologic alterations as accelerating factors in the development and occurrence of CVD [1][3][5][10]. The whole blood viscosity as an index of blood fluidity is a complex phenomenon including plasma, erythrocyte, platelet, and leukocyte components [1][2]. Impaired erythrocyte deformability, measured by the cell filtration technique, is believed to play a leading cause of the microcirculatory disturbances present in patients with CVD [1][4][6]. However, more recent studies have shown that blood filtration reflects not only erythrocyte deformability but also retention of leukocytes in the filter [9][10][11]. Early filtration methods could not differentiate between these two components influencing blood filtration. The recently described St. George's filtrometer is capable of differentiating between these two components by measuring both the initial filtration rate and erythrocyte transit time when filtering erythrocyte suspensions (Table 1). Using this technique, it has been shown recently that not only the erythrocyte but also the leukocyte filterability is changed in recent stroke [10][11][12]. Blood samples with and without standardized leukocyte counts were filtered, and a higher filter occlusion was found only if leukocytes were present in the samples. One can argue, however, that these changes are not specific for cerebrovascular disorders but only indicate nonspecific reactions of leukocytes during acute ischemic tissue damage. Therefore, we studied blood filterability in patients with chronic CVD in whom acute tissue damage, which can influence the properties of leukocytes, is definitely not present. By using a different approach and a different patient population we were able to confirm the observations of Ernst et al. [13]. Our data show a highly significant increase in filter clogging when erythrocytes with low but detectable leukocyte contamination or when suspensions with only leukocytes are filtered. We did not find changes in initial filtration rates during erythrocyte filtration, suggesting that the rheologic alterations are caused by leukocytes only. Accordingly, altered blood filterability in CVD as described earlier [15][17] may be a consequence of changes in leukocytes rather than in erythrocytes. Our results do not clarify which leukocyte subpopulation may account for these abnormalities or which pathophysiologic changes are involved in altered leukocyte rheology in these patients. We can only speculate that, in addition to a possible role of altered leukocyte rheology influencing the size of the infarct during the acute phase, it also is important as a prognostic factor in the chronic phase of CVD before the events. Experimental observations suggest that leukocyte rheology is potentially of greatest clinical significance in conditions associated with low blood flow or with hypertension [18]. We surmise that decreased deformability or increased adhesiveness of leukocytes or both demonstrated in chronic CVD patients may play a role in the chain of events leading to impairment of the microcirculation in CVD.

**References**


**Table 1. Filtration Results of Erythrocyte and Leukocyte Suspensions of Patients With Cerebrovascular Disease and of Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 28)</th>
<th>Controls (n = 30)</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>Erythrocyte suspension</strong> (packed cell volume 10%)</td>
<td></td>
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<tr>
<td>Initial filtration rate</td>
<td>0.522 ± 0.04</td>
<td>0.534 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Erythrocyte transit time</td>
<td>10.54 ± 1.48</td>
<td>10.33 ± 1.05</td>
<td>NS</td>
</tr>
<tr>
<td>Clogging particles (ml⁻¹)</td>
<td>28,535 ± 1,995</td>
<td>16,844 ± 1,139</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Leukocyte suspension (1 X 10⁹ cells/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial filtration rate</td>
<td>0.924 ± 0.02</td>
<td>0.943 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Clogging particles (ml⁻¹)</td>
<td>152,278 ± 14,852</td>
<td>96,935 ± 4,155</td>
<td>&lt;0.001</td>
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</tbody>
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Values are mean ± SD. Probability by Student’s paired t test; NS, no significant difference.

Key Words • cerebrovascular disorders • leukocytes • rheology
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