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Erythrocyte Rheology in Acute Cerebral Thrombosis Effects of ABO Blood Groups

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Fließeigenschaften der Erythrozyten bei Patienten mit akutem zerebralen Gefäßverschluß

Zusammenfassung. Innerhalb weniger Stunden nach Bildung eines Thrombus in einer Hirnarterie wurden bei 220 Patienten die Deformabilität and Aggregabilität der Erythrozyten untersucht. Die Filtration von gewaschenen Erythrozyten zeigte bei 27,5% der Patienten Veränderungen der Deformabilität. Die Filtration ungewaschener Erythrozyten ergab bei 78,6% der Patienten eine Zunahme der Aggregabilität der Erythrozyten. Die Berechnung der Korrelationskoeffizienten zwischen Filtrations-Index und Fibrinogenkonzentration, die bei 88,2% der Patienten erhöht war, weist darauf hin, daß Störungen der Aggregabilität nur bei Patienten mit den Blutgruppen A und B abhängig von Fibrinogen sind, während es sich bei den Blutgruppen 0 und AB um andere Plasmafaktoren handeln muß.

Schlüsselwörter: Hirnthrombose – Deformabilität und Aggregabilität der Erythrozyten – Blutgruppen

Summary. Within a few hours after a cerebral thrombosis in 220 patients, the flow-properties of the red blood cells (RBC) were analyzed by a filtration test that expresses quantitatively the deformability and aggregability of the RBC by the filtration indexes pT. Abnormal deformability of the RBC washed clean of plasma was found in 27.5% of the patients. Aggregability disorders, caused by the plasma trapped between the unwashed RBC, were found in 78.6% of the patients: computation of correlation coefficients between pT indexes and fibrinogen, which was found abnormally high in 88.2% of cases, demonstrated significantly that in the patients with A and B blood groups these aggregability disorders were due to fibrinogen and that they were caused by other components of plasma in patients with O and AB blood groups. All these disorders can account for blood hyperviscosity.

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Key words: Cerebral thrombosis – Erythrocyte deformability and aggregability – Blood groups

In cerebrovascular diseases, high viscosity of blood has been found in a variable number of patients [15,16,28], and it was considered that this is due to the high plasma fibrinogen associated to a moderately increased hematocrit [15]. However, this may not be a sufficient explanation, since even more important for blood hyperviscosity are the flow-properties of the erythrocytes [5,6,11]. Consequently, we investigated the deformability and aggregability of the red blood cells (RBC) in patients with recent thrombosis of the cerebral arteries. In addition, we investigated the effects of the ABO blood groups on the correlation between RBC aggregability and fibrinogen, because there is evidence that: (1) thrombotic diseases are associated with particular blood groups [1,2,10,20,26]; (2) in cerebral thrombosis (and in cerebral hemorrhage) such an association is probable [18]; (3) the blood groups can influence the rheological aspects of thrombus formation [11].

Clinical Cases

We investigated 220 patients hospitalized for cerebral thrombosis of sudden onset; they had clear-cut neurological symptoms (e.g., hemiplegia or severe paresis, aphasia), and clear cerebrospinal fluid. We confirmed the diagnosis by careful follow-up of the patients; furthermore, we investigated 45% of them by cerebral arteriographies, which had positive results, and we confirmed the diagnosis by autopsy in the 43 deceased in the hospital. We did not include patients with transient ischemic attacks, those suspect of cerebral embolies, and those with cardiac disturbances (heart failure, atrial fibrillation, heart murmurs, pulmonary-artery stasis, abnormal electrocardiograms, etc.) that could cause hemodynamical disorders of the circulation: thus, one can reasonably assume that all 220 patients had an arterial obstruction by intravascular thrombus.

Methods

Blood samples were collected by venous puncture within a few hours after the stroke (never later than 36 h) and analyzed 1–2 h after collection. We investigated the flow-properties of the RBC by the erythrocyte filtrability test (FT), as described in detail by Teitel [29–31]. In brief, citrated blood was centrifuged at 600 g and the plasma, white cells and platelets were removed by pipetting. Two milliliters of packed erythrocytes (hematocrit > 90%) were filtered through a paper filter, driven only by the pressure exerted by their weight. The filtered volumes were measured over time, and the RBC filtrability was expressed by the index pT, which is the logarithm of the half-time of filtration. We performed the two variants of this test: FTW, in which the packed RBC are washed twice by successive suspension and centrifugation with isotonic saline; thus, we eliminate the trapped plasma, and therefore this variant indicates exclusively the flow-properties of the RBC, which depend on their deformability [11,19,21,29]. FTU, done with unwashed RBC, in which their filtrability is influenced by the plasma trapped between them; thus, beside the RBC deformability, this variant measures the RBC aggregability.

We established in 2,500 healthy blood donors that in the FTW-variant the normal pT index had a mean value of 0.61, with a standard deviation (SD) of 0.19. In the FTU-variant the mean value of the pT index was 0.72, with 0.18 SD. These mean values of the pT indexes are different with $p < 0.001$. We used them as controls for our patients, considering as pathological the pT indexes that are over 0.80 for FTW and over 0.90 for FTU.

In addition, we measured the plasma fibrinogen by the clot weight method described by Denson [9] and established the ABO blood groups of the patients by the classical method.

Results

The blood from the 220 patients had the following groups: 0 = 71 (32.2%; A = 92 (41.8%); B = 45 (20.4%); AB = 12 (5.4%). These percentages are not different from the general distribution of blood groups in this country, nor from those found in people deceased by cerebral thrombosis [18].

The fibrinogen concentrations were high, as it was also reported by Eisenberg [15], the mean value being 6.34 g/l (SD 1.84); in 88.2% of the patients fibrinogen concentration was higher than 4.50 g/l, which is usually considered as the maximum-normal value.

The filtration indexes pT found with the FTW test are shown in the frequency distribution diagram in Fig. 1. 27,5% of the patients had pT indexes higher than the maximum-normal of 0.80. We could not find any difference among these data when we took into account the blood groups of the patients.

The frequency distribution diagram of the pT indexes found with the FTU test (Fig. 2) showed that the plasma trapped between the unwashed RBC causes pathological alterations of the RBC aggregability in 78.6% of all patients, with values of the pT indexes higher than the maximum-normal of 0.90.

In the whole group of patients the analysis of linear regression revealed that the fibrinogen is one of the plasma factors that influence the alterations of RBC aggregability (Table 1). However, the correlation between fibrinogen and pT indexes is weak (Fig. 3), having a coefficient r of only 0.31 (with a probability $p < 0.001$ this r value cannot be, by chance, less than we have computed it in our sample). When we took into account the blood groups of the patients, the correlation was strong in the 92 patients of blood group A, with a coefficient $r = 0.80$ ($p < 0.001$), and in the 45 patients of blood group B, with the coefficient r of 0.84

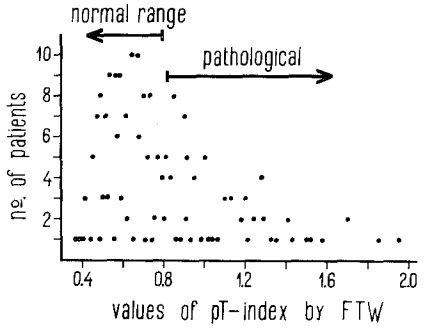

Fig. 1. Frequency distribution diagram of the values of pT indexes found in all 220 patients with FTW (Test of the filtrability of washed erythrocytes); the maximum-normal value of pT = 0.80

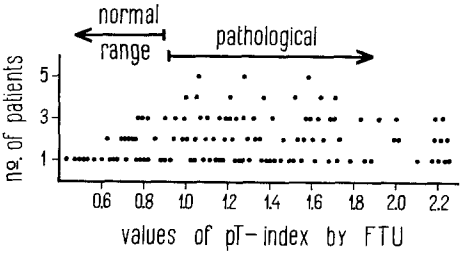

Fig. 2. Frequency distribution diagram of the values of pT indexes found in all 220 patients with FTU (Test of the filtrability of unwashed erythrocytes); the maximum-normal value of pT = 0.90

Table 1. The correlation coefficients r between pT indexes by FTU and fibrinogen and the ABO blood groups of the patients

No. of patients	Blood groups	Correlation coefficients r	$p <$
92	A	0.80	0.001
45	B	0,84	0.001
71	O	0.27	0.05
12	AB	0.26	—
Total 220	all groups	0.31	0.001

$p =$ with this probability p, this height of the correlation coefficient r cannot be less, by chance, in the respective statistical general population of patients

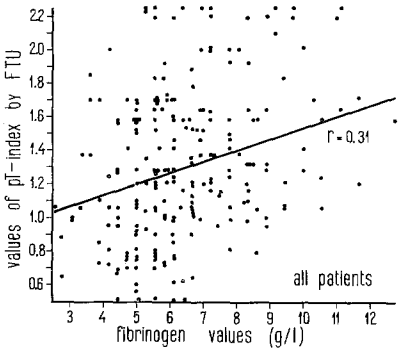

Fig. 3. Diagram of the correlation between the values of fibrinogen and those of the pT indexes by FTU in all 220 patients

($p < 0.001$). By applying the statistical r/z transformate of Fischer [32], that tests differences of significance between two correlation coefficients, we found that these correlations, for the blood groups A and B, are equally strong. On the other hand, the correlation was very weak in the 71 patients of blood group 0, with the coefficient $r = 0.27$ ($p < 0.05$) as well as in the 12 patients of blood group AB, with the coefficient r of 0.26; in this latter group, the number of only 12 patients is not sufficient for r to reach statistical significance. All the above shown probabilities p ascertain for the correlation coefficients r that the heights of their values cannot be less than they resulted by the respective computations (certainly, when one takes also into account the number of the cases in the investigated sample): thus, on the one hand, the highly significant $p < 0.001$ ascertains that the high correlation coefficients r found in blood groups A and B (0.80 and 0.84, respectively) are with certitude that high, while, on the other hand, the low probability $p < 0.05$ for blood group 0, and the lack of any statistical significance for blood group AB, indicate that the small values of their correlation coefficients (0.27 and 0.26, respectively) could possibly be in fact even less than we have found.

Discussion

We have considered that if in patients with acute cerebral thrombosis we investigate the blood as soon as possible after the stroke, we could demonstrate possible pathological alterations of those flow-properties of the erythrocytes, which are the most important for the viscosity of the blood [6, 11], viz., the disorders of RBC de-

formability [8, 17, 21, 27, 33, 34], and those of RBC aggregability [5, 14, 23]. Aggregability depends to some extent on RBC deformability, but even more on plasma factors, among which the fibrinogen is considered to be the most important [5, 14, 23], because it influences the RBC aggregability by the physical mechanism of macromolecular bridging between the cells [5, 13, 22]; on the other hand, fibrinogen also influences directly the blood viscosity, because it is the most important factor for plasma viscosity, which is increased when fibrinogen concentrations are high [5, 14, 24, 25].

It is considered [3, 21, 29] that with FTW only the RBC deformability is measured. However, the two washing procedures of the RBC do not rule out that a certain amount of plasma proteins could remain adsorbed on the RBC membranes and thus influence the RBC filtrability.

From our data one can accept that in no more than 27.5% of our patients there are pathological alterations of RBC deformability, which could account for a rise of the viscosity of their blood. In the majority of the patients (78.6%) disorders of RBC aggregability, as demonstrated by the FTU test, were present. Because also in the majority of the patients (in 88.2%) we have found abnormally high concentrations of fibrinogen, we have supposed that these might be the primary cause of the disorders of RBC aggregability, by an excessive macromolecular cell-cell bridging. However, the analysis of linear regression showed that the disorders of RBC aggregability were caused only to a small extent by the high fibrinogen concentrations, for the correlation coefficient between fibrinogen and the pT indexes was weak. This demonstrates that the disorders of the RBC aggregability, which we have found, are due only in a limited number of the patients to fibrinogen, while in the rest of them they must be due to other components of the plasma. In cerebral thrombosis the cell-plasma molecular relations are more complicated than it could be considered according to the known data [5, 14, 23]: our results demonstrate that the complexities are associated with the ABO blood groups of the patients, as shown below.

When the patients were separated according to their blood groups, the analysis of linear regression revealed that the fibrinogen causes the disorders of RBC aggregability only in blood groups A and B patients, as it was demonstrated by the strong correlation coefficients (0.80 for blood group A and 0.84 for B). These strong coefficients also show that in these blood groups the RBC membranes adsorb the plasma fibrinogen on a number of sites which is almost in a linear relation to the number of fibrinogen molecules. On the other hand, the RBC aggregability disorders in the patients with blood group 0 (and, perhaps, also AB), which are not caused by fibrinogen, as it is shown by the weak correlation coefficient ($r = 0.27$), must be due to other components of the plasma. These could be either the globulin fractions, which are also known as capable to influence the RBC aggregability [5, 7, 8, 21], or the alterations of the ion-concentrations, which could also modify cell-plasma molecular interactions [35]. Moreover, because even this small (0.27) latter coefficient is statistically ascertained only by a low significance p ($p < 0.05$), it is possible that in the general population of patients this coefficient could be even less than that found in our sample: obviously, this possibility would enhance the significance of all the above shown differences between the patients of blood group 0 as opposed to those of blood groups A and B.

We consider that the above shown cell-plasma relations are part of the blood syndrome that had caused the thrombosis, and that they are not non-specific alterations [4, 12] which had appeared as a secondary reaction of the blood to the injury of the brain, because in a preliminary investigation of 55 patients with a cerebral stroke due to acute cerebral hemorrhage we found no correlations between the pT indexes by FTU and fibrinogen ($r = 0.09$).

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