Russian Journal of Biomechanics, № 1: 37-46, 1999.

CHARACTERISTIC PARAMETERS DERIVED FROM WHOLE BLOOD VISCOMETRY IN CEREBROVASCULAR DISEASE

N. Antonova*, I. Velcheva**

*Institute of Mechanics and Biomechanics, Bulgarian Academy of Sciences, Akad. G. Bonchev Str., Bl.4, 1113 Sofia, Bulgaria

**Department of Neurology, Medical University, Tzarigradsko shosse, 4 km, 1113 Sofia, Bulgaria

Abstract: Nineteen controls and twenty one patients with cerebrovascular disease have been investigated with the Contraves LS 30 rotating cylinder viscometer and laboratory done capillary viscometers of Ubbelohde type. The present investigation uses the rigidity number "h", defined by the "Tokyo" expression of R.B.Whittington and J.Harkness [18], which itself incorporates both absolute and relative viscosity, as well as examination of the plasma viscosity. The results can basically be divided into two components: a viscous one (A) and an elastic non-Newtonian one (β). These are constant characteristics of the blood sample, quite independent on the hematocrit and on stresses imposed upon the blood during measurement. When the transferred in X and Y components are plotted, two distinct curves, patients' and controls' results touch. They are presented in a domain, suitable for indicating the difference between viscous and elastic forces. The blood rigidity number "h" as a function of plasma viscosity with evident periodicity is determined. A concentration range of blood cholesterol regarding ("h" - blood cholesterol) dependence in which h increases rapidly is found. The larger number of patients, diagnosed as being positive for cerebrovascular disease are confirmed by a criterion, including viscosity and rigidity number "h".

Key words: blood viscosity, plasma viscosity, blood rigidity number, cerebrovascular disease

Introduction

Our recent interest in hemorheological factors in cerebral ischemia has resulted in study of whole blood viscosity, its determinants as plasma viscosity, hematocrit and other biochemical parameters. The results of 38 patients with cerebrovascular disease (CVD) [1] show that the viscosity fell by about nine orders of magnitude, measured at rates of shear between 0.0237 sec^{-1} and 128.5 sec^{-1}. Nevertheless, it was found, that at ALL rates of shear, the AVERAGE whole blood viscosity and algebraically "corrected" viscosity to a standard hematocrit was higher in the patients than in the controls. These results as published showed deviations too wide for diagnostic purposes, but suggested that, with refinements, blood viscosity measurements might be capable of revealing the small changes taking place in asymptomatic and transient ischemic disorders. Another study on hemorheological changes in 170 CVD patients and 80 controls revealed significant correlation for plasma viscosity and fibrinogen, and for hematocrit and cholesterol in the control group and also for hematocrit and triglycerides in the group with asymptotic CVD [17].

Whole blood viscometry used in the above mentioned study offered a series of blood viscosity measurements at various selected rates of shear, that are frequently "corrected" algebraically (in the case) or physically to a standard hematocrit. Our works more recently have been based on the "Tokyo" formula of Whittington and Harkness [18], which carries the possibility of separating the viscous drag-forces from the non-Newtonian elastic relaxation - effects and calls for the measurement of the plasma viscosity, which was not done in the

earlier works [19,20]. The characteristics found, invariant to changing of hematocrit and stresses, comprehensively mapped the entire viscosity-hematocrit-shear domain. A sensitive viscometric method for early CVD was suggested that enables to distinguish very slightly disturbed bloods from the normal ones. The results were based on our 11 patients and we needed many more results before the patients' curve can be anything more than provisional. Various other pathological conditions have been studied by the (Y,X) and (h, η_0) criteria, and reported to a meeting of the Haemorheology forum of the Royal Society of Medicine [8,9]. One such set of results comes from sickle-cell patients, with an average h of nearly 30 (see Materials and Methods). When some of these bloods were deprived of O_2 (*in vitro*) - in some cases reaching some 30% of O_2 saturation - values of h of 70 or more were reached. A previous study on using A and β - characteristic parameters derived from whole blood viscometry and "Tokyo" expression, revealed their potential utility in the diagnosis and therapy in patients undergoing minor surgery, claudication, sickle cell and myeloma [21].

Materials and methods

A group of 21 patients (16 women, 5 men, mean age 56.9±10.1 years) with CVD was investigated. According to the last classification of CVD [4] they were defined as having asymptotic CVD (5 patients), transient ischemic attacks (4 patients) and chronic cerebral infarctions (12 patients).The apparent viscosity of whole blood and hematocrit values were measured in all patients and the results were compared to a control group of 19 age-matched healthy persons. Also haemoglobin and some plasma and serum protein and lipid constituents: total protein, fibrinogen, total cholesterol and triglycerides were examined.

Whole blood viscosity was determined by means of a rotational viscometer Couette LS 30 Contraves at a steady flow. Blood was drawn from a cubital vein between 8 and 10 a.m. and heparinized (15 IU/ml blood). One-ml samples were estimated up to 3 hours after blood withdrawal at 37^0 C and at 11 shear rates (ranging from 0.0237 to 128.5 s^{-1}). Estimations of hematocrit were done from the same sample with microhematocrit centrifuge TH 12 with centrifugation for 5 min at 10 000 min^{-1}.

Plasma viscosity measurements were performed at 37^0 C by the gravity force capillary viscometers worked out in the laboratory [2]. We use our Ubbelohde results having, on average, about 0.03 mPa.s higher, than those of others.

Our results - average Ubbelohde	1.31 mPa.s	n=31
average Matrai-Dormandy	1.27 mPa.s	n=55
average Harkness-Chalker	1.29 mPa.s	n=73
average Matrai, Harkness	1.28 mPa.s	n=130 (last two)

In the groups totalling 128 "controls", the upper limit of Harkness-Matrai, was arbitrarily fixed at 1.40 mPa.s and we used in our work this upper limit.

Parallel with viscosity measurements some plasma and serum proteins and lipids were estimated: total protein by biuret method, fibrinogen - by coagulation method according to Clauss, cholesterol - by enzyme method with tests of Boehringer and triglycerides - by UV method also with Boehringer's tests.

The primary assumption, indeed, springs from the work of Einstein [5], who treated the viscosity of a particulate suspensions in terms of the disturbances created in the dispersion fluid by the presence of the particles. Whittington and Harkness presented the "Tokyo expression"

$$\eta / \eta_0 = \left\{ \frac{A}{(1 - \log y / \log y_c)^{\beta}} \right\} \varphi \qquad (1)$$

where φ is the HCT %; η is the whole blood viscosity (mPa.s); η_0 is the plasma viscosity (mPa.s); γ is the rate of shear (s^{-1}); γ_c is the critical low shear rate (s^{-1}) derivable from the manufacturer's data (see Materials and Methods); A is the "viscosity parameter"; β is the "shear-sensitivity exponent" which links the Non-Newtonian relaxation effect into the equ.(1) and is some orders of magnitude smaller than "A".

For the convenience of mapping with co-ordinates of similar magnitude, we write $X=10^3(A-1)$: so that $(Y-X)=h=10^3(A-\beta-1)$, the physical attributes of A and β being transferred to Y and X, and that of the difference between the viscous and elastic parameters being transferred to "h".

Features of the "Tokyo Expression"

1) It is essential to begin with the relative viscosity η / η_0, in view of EINSTEIN's approach, mentioned above.
2) Removal of the non-Newtonian relaxation is obtained by setting $\beta = 0$, giving the simple exponential $\eta / \eta_0 = A^{\varphi}$ for suspensions of rigid particles.
3) Thyxotropic behaviour results when $\beta < 0$.
4) When $\gamma = \gamma_c$, $\eta/\eta_0 \rightarrow \infty$, the driving force becomes impossible large.
5) $1 - \log \gamma / \log \gamma_c$ is an arbitrary presentation of the applied shear-strain.
6) A and β are solved for two widely - spaced rates of shear in Eq.(1).

The value $\gamma_c = 1$ is precisely forbidden by the form of rate - function, since β temporally disappears from the equation.

The significance of the number h is shown in Fig.1, where our extreme values for the

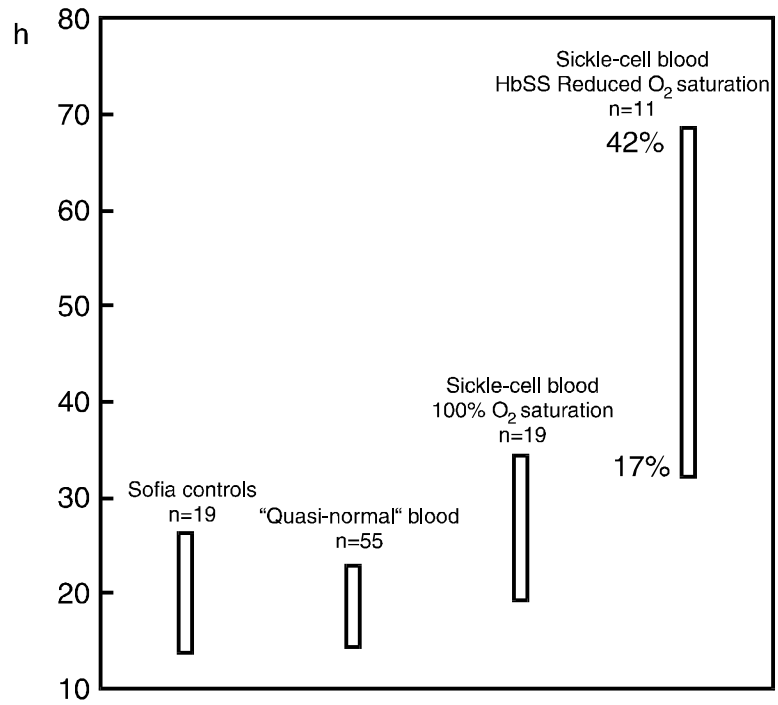

Fig. 1. "Blood rigidity number" in normal and sickle bloods.

"Sofia" controls average about h=17. The physico-chemical effects of the O_2 deprivation were shown in the experiments of Hussain [8,9] (King's College Hospital, London), expressed in large, but irregular increase in h, up to value of about 70. As a result of these experiments R.B. Whittington named h as the "blood rigidity number". Fig.1 is self-explanatory, showing the very large values of "h", which are attained in sickle-cell blood, especially when to abnormalities of cell shape and size are added the rigidity which occurs when the Hbs tend to form a gel at 37^0 C.

Critical Rate of Shear

Each speed at the rotational viscometer LS 30 Contraves is designed by a "speed number", N, which increases in such a way that a shear rate is given by:

$$\gamma = \gamma_c . 10^{N/7.5}$$

where γ_c is a lower limiting shear rate characteristic of the instrument and has the average value 0.012849 s^{-1} [3] for the standard dimensions of the suspended "bob".

Results and discussion

The results of 21 patients with CVD show that the viscosity is elevated over the whole range of shear rates in comparison with the control group of 19 healthy subjects. It should be noted that standard deviations from the mean values of the apparent viscosity reached up to 50% from these values at low shear rates, plasma viscosity is within normal ranges (Table I).

Table I.

The mean whole blood (η) and plasma (η_0) viscosity values of the investigated groups and their standard deviations, determined by LS30 Contraves at 37^0 C.

γ, s^{-1}	η, mPa.s	
	19 controls H=42.19 ± 3.86 %	21 patients H = 43.93 ± 4.2 %
0.0237	71.9 ± 51.45	98.46* ± 51.66
0.0596	42.99 ± 38.65	53.51* ± 26.26
0.1102	32.56 ± 21.81	44.33* ± 27.28
0.512	18.57 ± 8.6	25.25** ± 8.4
1.285	13.51 ± 5.55	17.95** ± 5.31
5.96	7.77 ± 2.68	10.18*** ± 2.46
11.02	6.44 ± 1.91	8.34*** ± 1.82
20.4	5.66 ± 1.38	7.03*** ± 1.51
51.2	4.72 ± 0.93	5.65*** ± 0.92
94.5	4.25 ± 0.74	4.98*** ± 0.76
η_0	1.33 ± 0.05 mPa.s	1.32 ± 0.04 mPa.s

*$p \leq 0.3$ **$p < 0.02$ ***$p < 0.006$

The mean values of biochemical parameters in patients with CVD are as follows: hematocrit - 42.19±4.2%; fibrinogen - 2.895±0.52g/l; cholesterol - 6.29±1.84mmol/l;

triglycerides - 1.13±0.71; total protein - 72.19±8.9 g/l. They show no significant differences in comparison to controls.

In seeking the diagnostic test we assume that the parabolic curve divides the X,Y plane into two major regions:

(a) PATHOLOGICAL to the left of and above the curve. Curve less than experiment, error negative;

(b) Normal to the right of and below the control line.

A certain "scatter" must be allowed for in both curves, with points slightly to the right of the parabola requiring careful scrutiny (+ve errors).

As shown on the Figure 2 (the X,Y plane), the patients' curve and the controls' line touch where the slope of the patients' curve equals 1.25. The patients' curve is for 21 cases, one of which, No.11, may require confirmation of the diagnosis.

A useful equation for a constant positive Y for patients is:

$$Y = 1/2\ aX^2 + bX + c, \qquad (2)$$

where a=0.175; b=-6.59; c=183
and for the control line is:

$$4Y = 5X + 30. \qquad (3)$$

A basic difference between the curves is that in the patients' curve, the second derivative Y" is always positive: (Y'=aX+b; Y"=a), whereas in the control line, Y" is always zero: (Y'=5; Y"=0). Neither the patient nor the control line are explicit, but, it is clear that the first derivatives will equalise between Y = 60 to 65.

Average of all ΔY (excluding No.11) = 0.166±0.43; 3/0.166 = 18.07 . Thus the

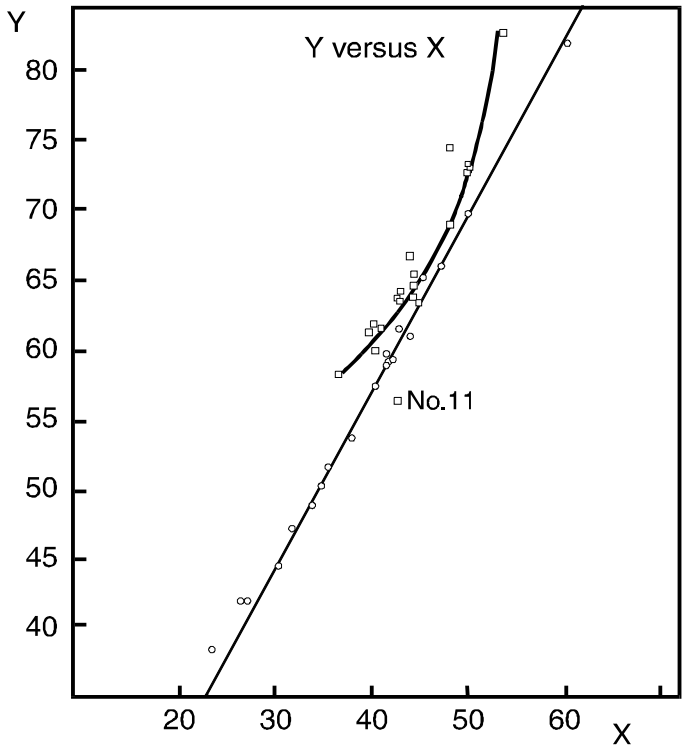

Fig. 2. Patients' curve and the control line in the X,Y plane ; □ - measured points from patients with CVD, o - measured points from healthy donors.

deviation of No.11 Y+ is more about than eighteen times average ΔY+. Since there is inevitable "scatter", the area between curve and line is of doubtful meaning.

The curve is very steep as we approach patient 10; therefore the error in Y becomes very large. The error in X is only about -2.

Patients with negative errors (experiment greater than curve) must be regarded as pathological; and such patients with negative errors, as -4.18 (No.10) and as -5.7 (No.14) belong to the pathological region.

TABLE II.

Absolute values of the formula error in Y = Δ Y of the twenty one patients.

No.	1	2	3	4	5	6	7	8	9	10	11
Y_{exp}	59.9	62.69	58.18	63.63	68.93	61.31	64.54	59.56	63.81	72.78	61.14
$Y_{form.}$	59.96	64.47	58.96	63.69	67.97	59.61	62.61	60.27	61.41	68.60	64.14
$X_{exp.}$	41.10	45.62	36.95	45.04	47.83	40.47	44.15	41.58	42.99	48.18	45.38
error	+0.06	+1.78	+0.78	+0.06	-0.96	-1.70	-1.93	+0.71	-2.40	-4.18	+3.00
No.	12	13	14	15	16	17	18	19	20	21	
Yexp.	66.88	65.34	69.02	68.66	66.55	66.49	61.20	60.88	62.03	63.11	
Yform.	65.66	64.24	63.98	67.64	63.11	65.75	60.79	59.53	61.18	60.93	
Xexp.	46.44	45.46	45.26	47.64	44.58	46.49	42.28	40.3	42.74	42.45	
error	-1.21	-1.1	-5.7	-1.02	-3.44	-0.74	-0.41	-1.36	-0.85	-2.18	

TABLE III.

X,Y -values of the nineteen controls

No.	1	2	3	4	5	6	7	8	9
$Y_{exp.}$	42.5	46.0	42.5	47.5	52.0	77.3	58.5	59.4	61.5
Xexp.	28.0	30.5	27.9	31.6	35.6	51.8	41.7	42.0	43.2
No.	10	11	12	13	14	15	16	17	18	19
Yexp.	54.1	60.9	59.9	61.2	38.1	49.6	65.3	66.7	57.4	57.51
Xexp.	39.1	45.2	43.6	42.1	22.3	34.4	46.3	48.5	40.8	41.33

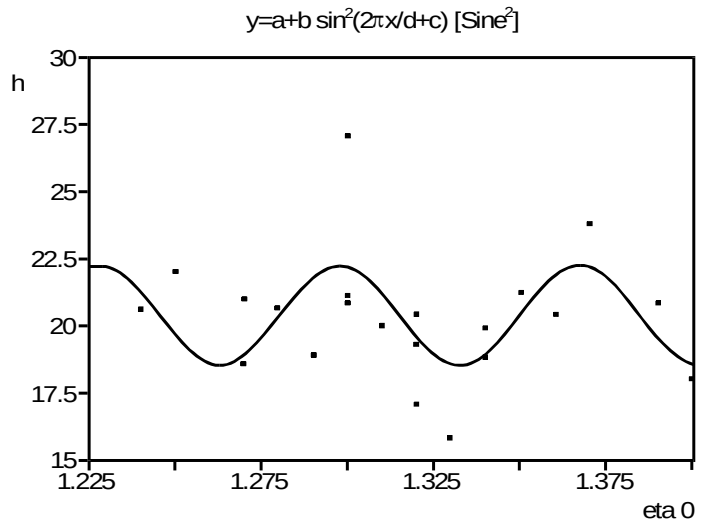

Fig. 3. h = Y-X = Rigidity number as a function of plasma viscosity (η_0).

We turn to analyse a different pair of variables h-η_0. An unsuspected periodicity of this arrangement of the patients' points was found. The Figure 3 (h,η_0 plane) gives the answer for the kind of this function - squared sine function:

$$h = a + b \sin^2(2\pi \eta_0/d + c) \qquad (4)$$

with coefficients: a = 18.5; b = 3.73; c = 3.79 and d = 0.139 (residuals r^2 = 0.349; FitStdErr.=2.028, Fstat = 3.038). Patients with large negative errors (Table II) as -4.18 (No.10), -5.7(No.14) and No.11 (3.00) is shown to be out of the group of this periodic arrangement of the points.

Figure 4 (h versus blood cholesterol) throws a different light upon the significance of

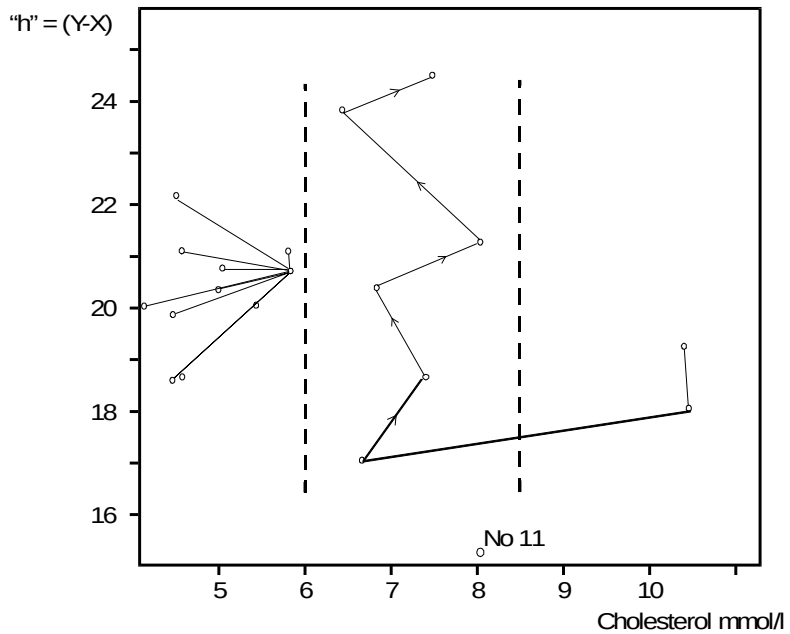

Fig. 4. Three ranges of alteration of h with cholesterol concentrations for cerebrovascular disease.

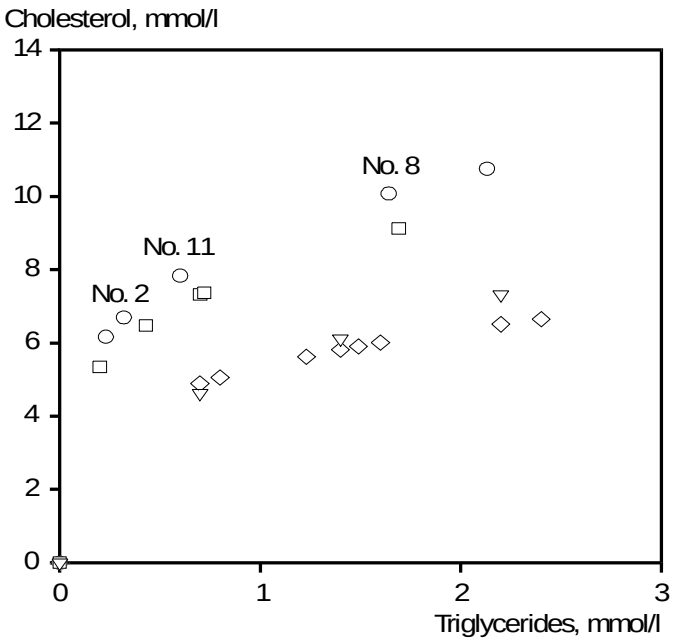

Fig. 5. The relationship between blood cholesterol and triglycerides for the 21 patients.

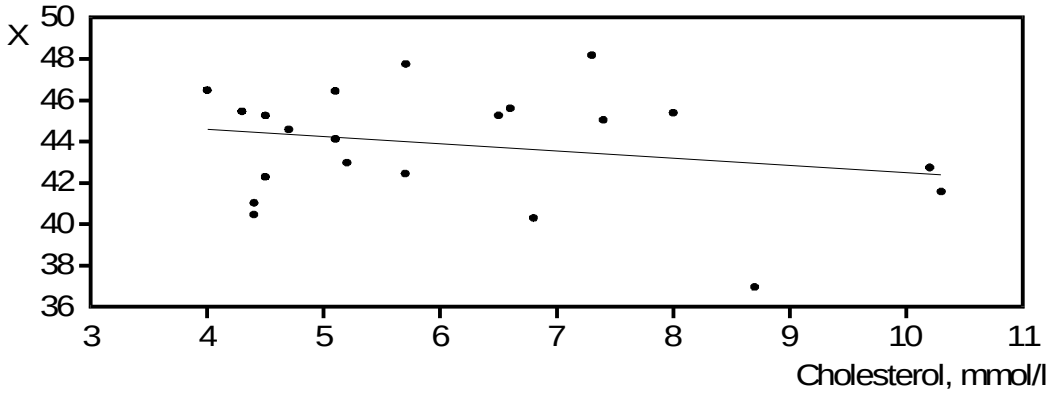

Fig. 6. The regression line presents the effect of cholesterol on X for the 21 patients.

h and suggests three ranges of its alteration with blood cholesterol concentrations. The first range (4÷6 mmol/l) in which h is about the mean value for the patients' group (h=20.29). The second concentration range (about 6 to 8 mmol/l) in which h increases rapidly from about 17 to about 27.01 with small changes in cholesterol level, i.e. the rigidity increases rapidly by about 50 %. The third concentration range (h ≥ 6 mmol/l) in which h is about 18. The patient No.11 has the lowest h values in the second concentration range. The information which we have available proposes that there are other biochemical contributions of blood-cholesterol to the purely physical picture which we are building up with our viscometers.

The biochemical observations on CVD patients show that cholesterol-total glycerides relations could be defined by the formula: Chol = constant . $(Trg)^{1/4}$, illustrated in Fig.5. Figure 5 shows that Cholesterol abnormalities may account for the values with positive errors (of No.2, No. 8 and No.11 - Table II). The biochemical test is verifying the fact, that in the patients there is some kind of equilibrium between cholesterol and total glycerides. The relation between X and Cholesterol shows, that, on the whole, X decreases with Cholesterol. This implies that on the whole the bad effect of Cholesterol is to reduce increase in Non-Newtonian relaxation (Fig.6).

It is well known that blood and plasma viscosity are strongly dependent on hematocrit and on the plasma levels of fibrinogen, cholesterol and triglycerides [12]. These plasma constituents seem to correlate independently with plasma viscosity. So Jung et al. [10] reported the leading influence of fibrinogen in determining plasma viscosity. Independent influence of elevated blood triglycerides on plasma viscosity over a broad range of shear rates after adjusting for fibrinogen was shown by Rosenson [14] . For blood viscosity the influence of fibrinogen predominated over lipoproteins. D. Eterovic et al. [7] found that expressed in standardised residuals the effect of cholesterol was about three times greater compared to fibrinogen and triglycerides. In their study the leading influence of cholesterol on plasma viscosity was delineated. Our data also confirm the effect of cholesterol on the blood viscous properties. The importance of this finding increases even more, since the role of cholesterol as risk marker for CVD is less well defined in comparison to other stroke risk factors.

The rheological and lipid parameters, determinants of whole blood and plasma viscosity have been studied as risk factors for CVD. Prospective epidemiological investigations indicate plasma fibrinogen as independent predictor of myocardial infarction and stroke [15]. A number of case controlled epidemiological studies has shown no significant relation of total plasma cholesterol to the risk of stroke [13]. However a contemporary overview of 10 prospective trials on the relationship between plasma cholesterol values above 5.7 mmol/l and the risk of CVD showed a cumulative risk of 1.31, which is statistically significant [13]. A recent study on the influence of total cholesterol on the risk of CVD revealed a non-linear relationship with significant increase of the stroke risk

only for the cholesterol values above 8 mmol/l [21]. The data on the link between triglycerides and CVD are also controversial.

Conclusion

The results of 21 patients with CVD show that the viscosity is elevated over the whole range of shear rates in comparison with the control group of 19 healthy subjects. A sensitivity method, distinguishing very slightly disturbed bloods from the normal ones was searched. The larger number of patients diagnosed as being positive for cerebrovascular disease were confirmed by a criterion, including viscosity and rigidity number h. In X,Y domain two distinct curves - patients' and controls' result. A much more complicated relationship between "Blood rigidity number h" and plasma viscosity of periodic kind was found.

To the purely physical picture build up with our viscometers other biochemical contributions - of blood cholesterol and triglycerides - were added. A concentration range of blood cholesterol was proposed in which "Blood rigidity number h" increases rapidly with small changes of cholesterol.

Acknowledgement

This work was done under supervison of Dr. R. B. WHITTINGTON and further developed after his death. It was supported by the National fund for Scientific Investigations - Ministry of Education and Science, Sofia, project grant No. L-309.

References

1. ANTONOVA N., VELCHEVA I., BOEVA D. Hemorheological disturbances in Ischaemic Cerebrovascular Disease. **Biomechanics,** 24: 28-33, 1990.
2. ANTONOVA N., VElCHEVA I., BOEVA D., MARINOVA T.S.V. Comparative plasma viscosity measurements in healthy subjects. **Clinical Hemorheology**, 13: 45-52, 1995.
3. BLACK R.A., HOW T.V., WHITTINGTON R.B. The calibration of rotational instruments for measurement of whole-blood viscosity. **Biorheology**, 23: 485-498,1986.
4. Classification of Cerebrovascular Disease III, **Stroke**, 21: 637-676, 1990.
5. EINSTEIN A. Eine neue Bestimmung der Molekule-dimensionen. **Ann der Physik**, 19: 289-306,1906.
6. ERNST E., RESCH K.L. Fibrinogen as a cardiovascular risk factor: A Meta-analysis and review of literature. **Ann Int Med**, 118: 956-963,1993.
7. ETEROVIC D., PINTARIC I., TOCILJ J., REINER Z. Determinants of plasma viscosity in primary hyperlipoproteinemia. **Clin Hemorheology**, 15: 841-850,1995.
8. FISHER A.C., WHITTINGTON R.B., HUSSAIN S.M. The blood - rigidity number in some normal and pathological states. Meeting of the Haematology Forum of the Royal Society of Medicine, London, Sept. 1990.
9. HUSSAIN S.M. On the viscosity of Sickle-cell blood. Private communication to R.B.W. 1989.
10. JUNG F., PINDUR G., KIESEWETTER H. Plasma viscosity dependence on proteins and lipoproteins: Results of the Aachen study. **Clin Hemorheology**, 12: 557-551,1992.
11. LINDENSTROM E., BOYSEN J., NIBOE J. Influence of total cholesterol, HDL-cholesterol and triglicerides on the risk of cerebrovascular disease. Copenhagen cardiovascular study, **BMJ**, 309: 11-15, 1994.
12. LOWE G.D.O. Blood viscosity, lipoproteins and cardiovascular risk (editorial). **Circulation**, 85: 2329-2331, 1992.
13. QUIZIBLASH N., DUFFY S.W. , WARLOW C., MANN I. Lipids are risk factors for ischemic stroke: overview and review. **Cerebrovasc Dis**, 2: 127-136, 1992.
14. ROSENSON R.S. Rosenson, Influence of triglycerides and fibrinogen on blood viscosity. **Clin Hemorheology**, 13: 365, 1993.
15. SINDHARAM R. Risk factors for ischemic stroke: a case control analysis. **Neuroepidemiology**, 11: 24-30, 1992.
16. UBBELOHDE L. In: **Handbuch d. Chemie und Technologie d. Cele**, I, 340 pp, 1908.

17. VELCHEVA I., BOEVA D. Hemorheological disturbances in cerebrovascular disease. **Cerebrovascular Diseases**, 1: 29-32, 1993.
18. WHITTINGTON R.B., HARKNESS J. Whole blood viscosity as determined by Plasma Viscosity, Haematocrit and Shear, **Biorheology**, 19: 175-184, 1982.
19. WHITTINGTON R.B. , ANTONOVA N. Viscometric test of early cerebrovascular disease. In: Stoltz J.-F., Zhuang Feng-yaun, Liao Fu-long and Potrong G. (Editors), **Proceedings of the First China-France Symposium on Biorheology**. Beijing, China, Aug. 31 - Sept. 2, 1994, 243-246.
20. WHITTINGTON R.B., ANTONOVA N. Blood viscosity measurements in the detection of early cerebrovascular disease. **Theoretical and applied mechanics**, 25: 1994-1995.
21. WHITTINGTON R.B., MATRAI A., NICOL C.G. Characteristic parameters derived from whole blood viscometry: their potential utility in diagnosis and therapy. (read at 5th Int. Congress of Biorheology, Baden-Baden, F.R. Germany,1983).

Характеристические параметры, полученные с помощью вискозиметрии цельной крови при церебральнососудистых заболеваниях

Н.Антонова, И.Велчева (София, Болгария)

Были проведены экспериментальные исследования вязких свойств крови у 19 здоровых и 21 больных пациентов с церебральнососудистыми заболеваниями. Для этого применялись капиллярные вискозиметры и вискозиметры с вращающимся цилиндром. В исследовании использовался параметр жесткости h, который включает абсолютную и относительную вязкость крови, а также вязкость плазмы. Эксперименты показали, что у больных пациентов наблюдается повышение вязкости крови во всем диапазоне скоростей сдвига. Применение метода чувствительности позволяет диагностировать очень малые изменения вязкости крови. Найдено также весьма сложное соотношение между параметром жесткости и вязкостью плазмы. Отмечается, что вязкость крови и параметр жесткости крови являются весьма полезными критериями для оценки церебральнососудистых заболеваний. Библ. 21.

Ключевые слова: вязкость крови, вязкость плазмы, параметр жесткости крови, церебральнососудистые заболевания

Received 06 August 1998