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Analysis of Haemorheological Variables—Methodology and Reference Values

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ABSTRACT

Routine methods are proposed for the analysis of haemorheologic variables with only one instrument, a Low Shear 30 rotational couette viscometer. The variables are plasma viscosity, whole blood apparent viscosity, erythrocyte aggregation tendency and erythrocyte fluidity.

These variables, in addition to erythrocyte volume fraction, were analysed in a population of 83 healthy hospital staff subjects.

The following means and standard deviations were found: Erythrocyte volume fraction, women 42±3 %, men 46±2 %; Plasma viscosity 1.31±0.07 mPa·s; Whole blood apparent viscosity, women 4.3±0.4 mPa·s, men 4.8±0.3 mPa·s; Erythrocyte aggregation tendency 1.00±0.10; Erythrocyte fluidity 122±10 $Pa^{-1} \cdot s^{-1}$.

INTRODUCTION

The flow of blood is substantially influenced by its rheology, an important variable which is changed in a great number of diseases, such as polycythemia and diabetes mellitus. Formerly, the interest of haemorheology was focussed only on the erythrocyte volume fraction (EVF) and the viscosity of plasma and whole blood. During the last two decades, however, the importance of erythrocyte deformability and aggregation tendency has been recognized. In the last ten years, a wide variety of different methods and instruments have emerged for the assessment of haemorheological variables but they are often tedious to use or expensive, and best suited for research work. Comparison of rheologic values from the literature is also difficult due to the heterogeneity in methodology and instrumentation.

The analyses of haemorheological variables have therefore been sparsely used in clinical work due to the lack of simple and uniform methods as well as reference values.

The aim of this study was therefore to develop simple and inexpensive but still adequate routine methods for the analysis of haemorheological variables with only one routine instrument, and also to investigate the distribution of these variables in a healthy population.

SUBJECTS AND METHODS

Subjects

The studied population comprised 83 healthy subjects, 19 to 65 years old (mean 38 years), mostly laboratory personnel but also technical and office hospital staff. Twentyeight were smokers and 29 were physically active (more than one physical training activity per week). Other characteristics are shown in Table 1.

Methods

Body mass index. Body mass index (BMI) was calculated as body weight divided by the square of body height (kg/m^2).

Blood sampling. Sampling was carried out in the morning without fasting regimen but after the subject had been sitting for 15 minutes in order to restore basal haemoconcentration. A tourniquet was used during the sampling from the antecubital vein into two 10-ml dry-heparinised vacutainer tubes (Terumo venoject). The tubes were inverted ten times and subsequently stored in a waterbath at +37 ^{o}C when not otherwise handled in the analysis procedure.

Analyses. EVF was analysed by microhaematocrit centrifugation at 11000 g for 5 minutes. No correction was made for trapped plasma.

All rheological analyses were carried out at +37 ^{o}C in a Couette rotational Low Shear 30 viscometer (Contraves AG, Zürich, Switzerland) with a 1-1 couette (bob-in-cup) system, described by Spinelli and Meier (8). A sample volume of 0.70 ml was sufficient for a single analysis. Except for the analysis of erythrocyte deformability, all assessments were completed within one hour. Standardization of the instrument was performed daily by the analysis of degassed pure water, which has a viscosity of 0.695 mPa·s at 37 ^{o}C (4).

Plasma viscosity (P-viscosity) was analysed at a shear rate of 38 s^{-1} and whole blood viscosity both at 100 and at 1 s^{-1}. The value of whole blood viscosity at high shear rate was taken to represent whole blood apparent viscosity (B-viscosity).

The whole blood viscosity at low shear rate is essentially dependent on erythrocyte aggregation, and therefore the erythrocyte aggregation tendency (E-aggregation) was derived as follows: the low shear viscosity value was first divided by the corresponding P-viscosity to obtain the relative apparent viscosity according to Chien et al. (2); then the relative apparent viscosity was divided, at the corresponding EVF-value, by the relative viscosity value from the EVF/relative-viscosity curve, established for all subjects (Fig. 1,

right). The unit for E-aggregation will consequently be dimensionless. The EVF/low-shear-whole-blood-relative-apparent-viscosity curve is approximately logarithmic and intersects the Y-axis at $\eta_{rel} = 1$ by definition (η = dynamic viscosity).

Erythrocyte deformability was assessed indirectly by the apparent fluidity at a shear rate of 0.95 s^{-1}. The erythrocytes were washed once and resuspended to an EVF of 55 % in isotonic saline-phosphate-glucose buffer (PBS) at pH 7.4 with a glucose concentration of 5 mmol/l. Deformability was expressed as erythrocyte apparent fluidity (E-fluidity) with the unit $Pa^{-1} \cdot s^{-1}$.

Procedure. One of the two obtained vacutainer tubes (tube A) was immediately centrifuged at room temperature for 10 minutes at 1400 g for the subsequent analysis of P-viscosity and E-fluidity. The other tube (tube B) was used for the analysis of EVF, B-viscosity and E-aggregation.

After the determination of P-viscosity, the remaining plasma and buffy coat were sucked off from tube A. Care was taken not to loose too many erythrocytes since the younger cells with lower density and higher fluidity will concentrate in the top layer. Then the tube was filled with PBS at +37 °C, capped and inversed until all sedimented cells were dispersed. After centrifugation as above, the supernatant was sucked off. Then the volume of packed erythrocytes was estimated and PBS was added so as to obtain an EVF of about 65 %. After thorough mixing as above, the EVF was analysed and a final volume of PBS was calculated and added. EVF was analysed again and accepted within the range 54 to 56 %. Deviations from 55 % were corrected for in the calculation of E-fluidity. Finally, the viscosity of the erythrocyte suspension was determined at a shear rate of 0.95 s^{-1}. The whole analysis procedure was completed within 120 minutes.

After thorough mixing of tube B, 0.70 ml of the whole blood was pipetted into the measuring cup, the bob was lowered and the speed of the cup was set to 72 r/min (shear rate 95 s^{-1}). After 90 seconds of temperature equilibration, readings were taken at shear rates of 95 and 129 s^{-1}. Then the speed was lowered to 0.72 r/min (shear rate 0.95 s^{-1}). The bob was moved up and down 2-3 times in order to remix cells and plasma and to randomize cellular orientation (7). Immediately after stabilization, readings were taken at shear rates of 0.95 and 1.29 s^{-1}. Viscosity values at 100 and 1 s^{-1} were derived by interpolation.

Statistical methods. Statistic probability was assessed by the Student's t-test on unpaired values. The error of a single determination of an analysis was calculated as the coefficient of variation (CV, per cent) from duplicate analyses according to the Dahlberg's formula: $CV = 100 \cdot \bar{X} / \sqrt{(\Sigma d^2 / (2 \cdot n))}$.

RESULTS

Characteristics of the subjects

There was no difference in age between men and women, but men had significantly higher BMI ($P<0.01$; Table 1).

Table 1. Mean, SD and range of age, body weight and height, and body mass index for both sexes.

	Number	Age (years)			Body weight (kg)		
		Mean	SD	Range	Mean	SD	Range
Women	48	37	10	19-62	60.2	6.9	47-75
Men	35	38	11	21-65	76.0	9.1	53-98
		Body height (m)			Body mass index (kg/m^2)		
		Mean	SD	Range	Mean	SD	Range
Women	48	1.66	0.07	1.52-1.80	21	2	17-26
Men	35	1.80	0.07	1.61-1.93	23	2	20-27

Haemorheological variables

The results of the haemorheological analyses are given in Table 2. EVF and B-viscosity are presented for both sexes due to the difference in EVF.

Table 2. Mean, SD, mean $\pm$ 2 SD, and CV of the haemorheological variables.

Variable	Unit	Mean	SD	Mean $\pm$ 2 SD	CV (%)
P-viscosity	mPa·s	1.31	0.07	1.16 - 1.45	5.4
EVF, F	%	42	3	36 - 48	6.9
EVF, M	%	46	2	42 - 50	4.0
B-viscosity, F	mPa·s	4.3	0.4	3.5 - 5.0	8.4
B-viscosity, M	mPa·s	4.8	0.3	3.8 - 5.8	6.9
E-aggregation		1.00	0.10	0.81 - 1.19	9.7
E-fluidity	$Pa^{-1}.s^{-1}$	122	10	101 - 142	8.1

Error of method and biologic variation

The error of the methods were calculated from duplicate analyses on 83 blood samples and expressed as coefficient of variation (CV) (Table 3). The rheological variables were also assessed on two consecutive days in 11 subjects (3 men and 8 women). This biologic variation (including the error of duplicate determination) is expressed as coefficient of variation (Table 3).

Table 3. Mean and CV of the methods (83 determinations in duplicate) and of day-to-day variation (11 subjects, 22 determinations in duplicate).

Variable	Mean value		Coefficient of variation (%)	
			Method	Total (method + biologic)
	n=83	n=11	n=83+22	n=11
P-viscosity, mPa s	1.31	1.30	0.7	3.1
B-viscosity, mPa s	4.5	4.4	0.9	2.6
E-aggregation	1.00	0.99	1.4	7.9
E-fluidity, Pa^{-1} s^{-1}	122	123	1.5	4.0

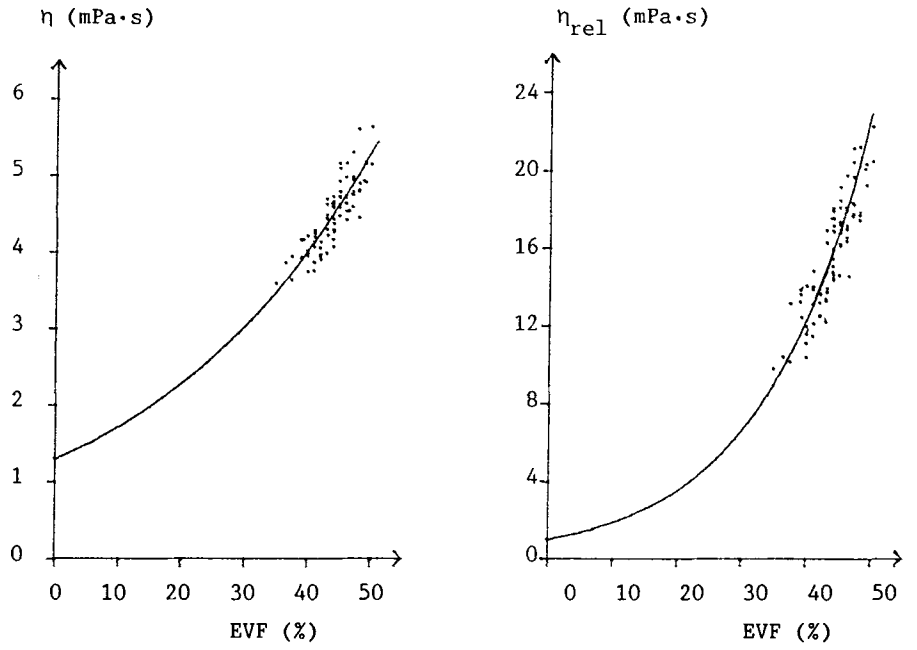

Fig. 1. Relationship between EVF and whole blood apparent viscosity. Left: whole blood apparent viscosity (η) at high shear rate (100 s^{-1}); Right: whole blood relative apparent viscosity (η_{rel}) at low shear rate (1 s^{-1}).

DISCUSSION

Routine methods were developed for the assessment of plasma viscosity, whole blood apparent viscosity, erythrocyte aggregation tendency and erythrocyte fluidity, with the use of only one couette rotational viscometer. The methods are simple to use and can be handled in a routine laboratory. An other important advantage in using only one instrument, a rotational viscometer, is that a newtonian liquid with known viscosity is sufficient for the standar-

dization of all analyses.

The relationship between EVF and whole blood apparent viscosity (B-viscosity) is approximately logarithmic and the curve intersects the Y-axis at $\eta \approx$ mean of P-viscosity (Fig. 1 left). A deviation in P-viscosity from normal will render an approximately parallel deviation from this curve. Therefore, the assessment of B-viscosity is primarily dependent on the EVF of the sample. The CV of the B-viscosity method (Table 3) was somewhat higher than that of the P-viscosity assessment. This fact underlines that it is vitally important to mix the whole blood thoroughly before the analysis of EVF and B-viscosity and also to draw samples for the two analyses from the same test tube.

The errors of the methods found in this study are low and in accordance with those found by Ernst et al (3) for the same instrument.

E-aggregation and E-fluidity were analysed at low shear rates, and the somewhat higher CV of these methods (Table 3) implies that the analytical error of the viscometer increases at lower shear rates. However, the determination of E-aggregation includes the rather complicated equilibrium between aggregation and disaggregation between the erythrocytes, which may have increased the analytical error.

In the assessment of E-fluidity, a preparation step precedes the viscometric analysis, and the CV was calculated on two assessments on the same preparation. The total error of the method was therefore higher than 1.5 % (Table 3). It ought, however, to be lower than the intraindividual day-to-day variation of 4.0 % including the error of duplicate analyses.

The reference values established in this study are used to validate the methods but can also be used as preliminary reference values in the screening of different diseases. The reference subjects represent an average population including both sexes, smokers and non-smokers, sedentary and physically active, obese and non-obese, and also a wide range of ages. The only criteria of exclusion were any disease (including common cold), and the use of drugs known to influence haemorheology, such as hypertensive drugs. Women using oral contraceptives were included, although these drugs have been reported to increase EVF and fibrinogen concentration and to impair E-deformability (1, 6). The reason is that this group is relatively large in the population.

When studying the haemorheology of a specific disease it is recommended to compare the patient group with a group of healthy subjects, matched for age, sex, BMI and smoking habits. Samples from the matched pairs should preferably be analysed on the same day in order to keep different factors constant, such as climate changes and instrument drift.

In the comparison between different methods or between laboratories, the International Committee for Standardization in Haematology (5) has recommended that the reference group should consist of young (20-30 years) healthy, non-

smoking, non-obese males that do not use medication or excess alcohol. The reason is that a very narrow distribution of the rheological variables can be expected in this group, which also presumably have unsignificant effects on the heamorheological variables due to age or drug abuse. Such a group will consequently be comparable between different laboratories.

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