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Comparison of blood viscosity using a torsional oscillation viscometer and a rheometer

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Abstract. The absence of a simple and clinically practical method to determine whole blood viscosity can partly justify why the medical community has been slow in realizing the significance of whole blood viscosity. For this reason, the availability of a technique able to evaluate blood viscosity in a rapid and direct manner is welcome. To evaluate the feasibility in hemorheological laboratory of a new torsional oscillation viscometer, it was compared with a conventional cone–plate system. The viscosity comparison has been related to hematocrit value both on whole blood and suspended blood in a saline solution. The results showed a good repeatability and reproducibility of the new equipment, with a best-fitting data of the hematocrit 0–100% range characterized by coefficient of determinations, $r^2 > 0.95$. Furthermore, a comparison of whole blood viscosity as measured by the two instruments was done on blood samples collected from hospitalized patients. Reasonable agreement for the viscosity values was found between the two methods with linear determination coefficients between the two measurement methods comprised between $r^2 = 0.7329$ and 0.9263, depending on shear stress phase and the corresponding shear rate.

Keywords: Blood viscosity, hematocrit, torsional-oscillation viscometer, rheometer

1. Introduction

The role of hemorheology in cardiovascular disease has been considered in the past by several authors, underlining the presence of blood hyperviscosity in acute and chronic disorders [1–4]. An alteration of blood viscosity, due to increase either of hematocrit (polycytemic hyperviscosity) or fibrinogen concentration (plasmatic hyperviscosity) or red blood cell rigidity (sclerocytemic hyperviscosity), is commonly considered a condition of high risk for acute or chronic brain ischemia [5–8].

In patients with spontaneous echo contrast (SEC) and atrial fibrillation (AF), we observed alterations of hemorheologic assessment with an increase of whole blood viscosity and fibrinogen that seems to be caused by an increase of red cells aggregability favoured by fibrinogen [9]. Same studies showed that modifications in endothelial function caused by physical stress are associated with a worsening in hemorheological parameters mainly in patients affected by ischaemic vascular diseases: major vascular alterations have been found in patients with very high levels of plasma markers endothelial dysfunction

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[10,11]. Viscosity levels, resulting from blood tests, depend on hematic properties (hematocrit, fibrinogen, erythrocyte deformability and erythrocyte aggregation) and on the blood test used [12,13]. Despite these aspects, medical community has been slow in realizing the significance of whole blood viscosity [14]. It can be partly attributed to the lack of easy and clinically practical methods of blood viscosity measurements. In most clinical studies, mainly two types of viscometer have been available for these purposes: rotational viscometers and capillary tube viscometer [15,16]. Recently, some studies have been carried out by viscometers, which are based on different measurement techniques: oscillating resonator [17,18] and torsional-balanced oscillation [19,20].

In particular, the purpose of our study is to evaluate the use and feasibility of the latter in a hemorheologic laboratory. For this reason, we compared this type of viscometer with a conventional rheometric cone–plate system, both studying the influence of hematocrit on viscosity changes and evaluating whole blood viscosity of blood samples collected from hospitalized patients. We wish the results of our study to be relevant to the field measurement protocols necessary for accurate use of these instruments, as well as the theoretical and clinical aspects of hemorheology.

2. Materials and methods

2.1. Torsional-oscillation viscometer

Viscomate VM-10AL (CBC Europe Ltd.). It is a torsional-oscillation viscometer characterized by constant shear stress systems driven by a piezoelectric ceramic source (Fig. 1). This instrument measures viscosity by sensing a change in oscillation amplitude of a liquid-immersed detector, based on constant input voltage. An original phase locked loop circuit maintains instrument resonant frequency of 1 kHz; the detector oscillation amplitude with no resistance is 1 μm. Angular acceleration of the detector is

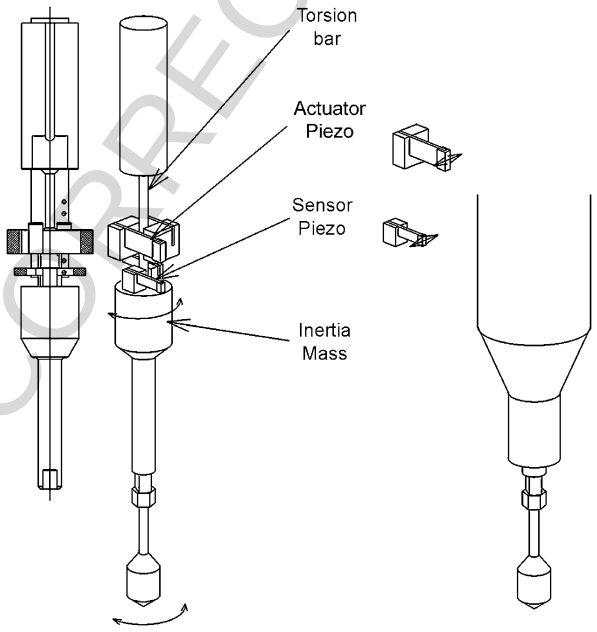

Fig. 1. Torsional oscillation viscometer – Viscomate VM-10AL (CBC Europe Ltd.).

measured and reported as dynamic viscosity with a declared range of 0.400–1000 mPa s and a precision equal to ±5%. The probe dimension was 9 mm with respect to the diameter.

All the determinations were conducted in polystyrene Technicon® sample cups (Kartell, nominal capacity 2 ml). In the case of stirring, PTFE micro magnetic stirring bars (3 × 3 mm) were adopted. A sample volume of 1.5 ml was used to determine blood viscosities. Temperature control was accurately monitored during the experiments (37.0 ± 0.2°C) by means of the sample cups into a water bath jacket (incubation time: 5 min). Viscosity values were recorded for six minutes (data collection every 5 s) by PC connection through a RS-232 port.

2.2. Rheometer

AR500 Rheometer (TA Instruments, Inc.). Cone–plate geometry (acrylic material, diameter 60 mm; cone angle 4°). The rheometer was previously calibrated with the viscosity standards. Viscosities values according to two steps, first the ascending shear stress phase (upward curve) and second the descending shear stress phase (downward curve) were obtained by TA Advantage software. In detail, after an initial equilibration phase, a linear continuous ramp with a starting shear stress of 0.02 Pa and an ending shear stress of 0.2 Pa was adopted for the upward step with a number of shear rate sample points equal to 90 for a duration of 1 min 30 s. At this point, the downward step was performed with the same modality (ending shear stress = 0.02 Pa; shear rate sample points = 90; duration = 1 min 30 s).

4 ml of blood were used to determine blood viscosity. Temperature control was accurately monitored during the experiments (37.0 ± 0.1°C) by a Peltier plate. No prevention loss of water due to evaporation was adopted. To exclude differences in personal style of using the instrument, measurements were always conducted by the same operator.

2.3. Blood collection

As far as blood viscosity determination as a function of hematocrit (Hct), blood samples of 150 ml were taken in the morning from two healthy, non-smoker, male blood donors, actually the authors (V.T., I.Z.). These samples were physiological and similar in terms of RBC count, fibrinogen level and other common hematological parameters. As far as instrument comparison, blood samples from informed hospitalized patients ($n = 70$; age > 60 y) were used. In all cases, blood using a 20 or 21 G needle with limited occlusion of the arm by the tourniquet was drawn. The blood was added to the EDTA anticoagulant (final concentration: 1.35 mg/ml), collection tubes with anticoagulant were gently inverted as soon after collection as possible to prevent clotting.

2.4. Sample preparations

To analyse the influence of hematocrit (Hct) on whole blood viscosity (η_{whole}), blood was centrifuged at 3000 rpm for 5 minutes at room temperature. Stock suspensions with a Hct of 0% and 100% were prepared by either adding or removing a calculated volume of plasma. To analyse the influence of fibrinogen and other non-cellular components on whole blood viscosity (η_{washed}) [21], blood was centrifuged at 3000 rpm for 5 minutes at room temperature to sediment red blood cells (RBCs), which were collected by discarding the supernatant. RBCs were then washed with saline solution four times to remove substances attached to the cell surface such as proteins, protein conjugate, polynucleotides, cell fragments, and small molecules. Then, RBCs were resuspended in saline solution to achieve a cell concentration range within 0–100%.

Hct determination and cellular concentration were obtained for each sample. Samples were not fully oxygenated when the Hct value was determined.

2.5. Mathematical fitting and statistical analysis

In the case of VM-10AL, the formula used to describe the dependence of viscosity on Hct and cell suspension is indicated in Eq. (1)

$$\eta = \eta_{\text{ref}} \cdot \mathrm{e}^{\mathrm{A}\cdot x}, \tag{1}$$

where η_{ref} represents either the plasma or the saline solution viscosity (37°C), as reported in the literature [22,23], A is a coefficient [24] and x represents the relative Hct values, expressed as percent.

In the case of AR500, Eq. (2) was adopted

$$\eta = \eta_{\text{ref}} \cdot \mathrm{e}^{\mathrm{A}\cdot x} + \mathrm{B}, \tag{2}$$

where B coefficient was also enclosed for taking into account shear rate and cellular volume fraction effects. For completeness' sake, B represents a simplified but useful correction factor, based on the overall theoretical consideration [25] overwhelming the aim of the present application study. Calculation was performed until we obtain the best fitting of the experimental points (as evaluated by coefficient of determination, r^2). Instat 3.0 and Prism 4.0, GraphPAD Software Inc., San Diego, CA were used.

As far as instrument comparison is concerned, whole blood viscosity values of hospitalized patients ($n = 70$) were determined and linear determination coefficients were evaluated, according to Eq. (3).

$$\eta_{\text{VM-10AL}} = m \cdot \eta_{\text{AR500}} + q. \tag{3}$$

3. Results

The results of the blood viscosity measurements *vs*. Hct as obtained by VM-10AL are shown in Fig. 2. To evaluate the influence, among other factors, of proteins and fibrinogen with respect to the viscosity,

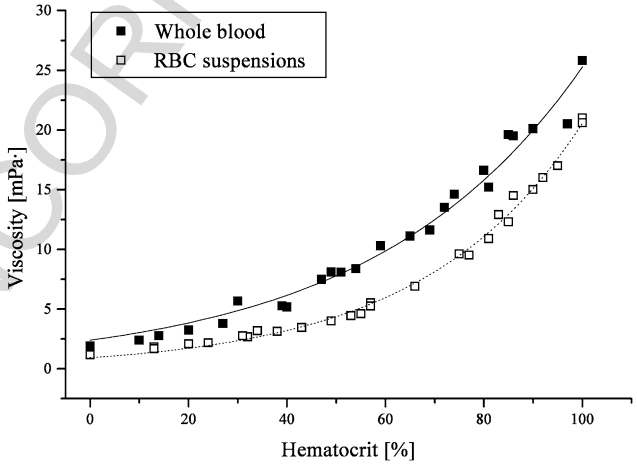

Fig. 2. Influence of Hct on blood viscosity measurement by torsional-oscillation viscometer (evaluable Hct range: 0–100%).

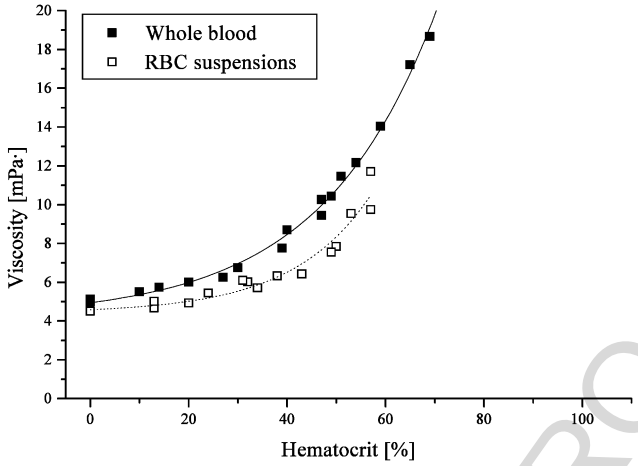

Fig. 3. Influence of Hct on blood viscosity measurement by AR500 at shear rate 10 s^{-1}, upward curve.

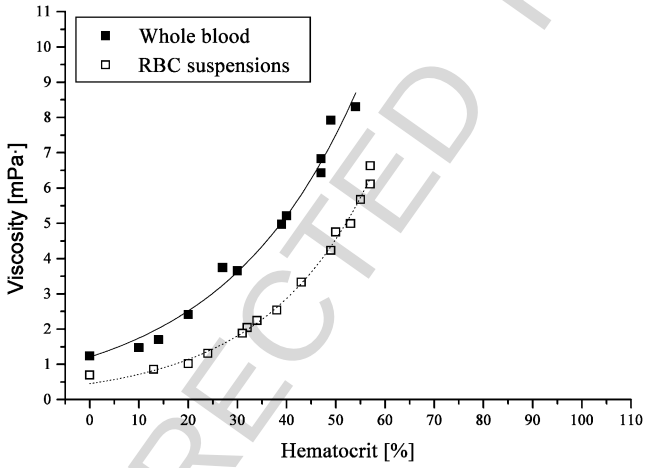

Fig. 4. Influence of Hct on blood viscosity measurement by AR500 at shear rate 25 s^{-1}, downward curve.

a series of concentrations and dilutions of red cells suspended in saline solution was also adopted. As it is possible to observe, viscosity values were obtained from all the tested Hct values. As expected, an exponential trend was evidenced in the case of both whole blood and RBCs resuspended in saline.

Figures 3 and 4 show the viscosity *vs*. Hct profile, as obtained by means of AR500 determinations at shear rate 10 s^{-1} of the upward step as well as 25 s^{-1} (downward step), respectively.

Also in these cases, an exponential behaviour was observed, but it was possible to obtain the viscosity values within a narrower Hct range, as reported in Table 1. However, it is not to be considered a limitation: in fact, the pathophysiological Hct interval was always covered, except than that is obtained for RBCs resuspended at downward step, shear rate 10 s^{-1} (Hct = 43–57, see Table 1).

In Figs 2–4, the lines are the best fitting curves obtained by using Eq. (2). The obtained coefficients of the best fitting parameters are reported in Tables 2 and 3 for whole blood and RBCs resuspended, respectively. For these analysis the value of plasma viscosity, $\eta_{ref} = 1.6$, and the value of saline solution viscosity, $\eta_{ref} = 0.7$, were adopted [22,23].

Table 1

Hct range useful for the determination of viscosity values, as obtained by AR500 rheometer

Hct range	AR500								
	Upward curve (s^{-1})								
	8	10	15	18	20	25	30	35	40
Whole blood	0–74	0–69	0–59	0–54	0–54	0–47	0–40	0–40	0–27
RBCs resuspended	0–57	0–57	0–57	0–57	0–57	0–57	0–57	0–50	0–43
	Downward curve (s^{-1})								
	40	35	30	25	20	18	15	10	
Whole blood	0–27	0–40	0–40	10–54	14–54	14–59	20–59	27–59	
RBCs resuspended	0–43	0–50	0–57	13–57	31–57	31–57	31–57	43–57	

Table 2

Coefficients for describing the dependence of whole blood viscosity (η_{whole}), as obtained by both VM-10AL and AR500, with respect to Hct, according to Eq. (2)

	VM-10AL	AR500								
		Upward curve (s^{-1})								
		8	10	15	18	20	25	30	35	40
A	0.028 (0.026)*	0.034	0.033	0.030	0.029	0.029	0.028	0.026	0.024	0.025
B	–	3.47	2.78	2.18	1.96	1.83	1.64	1.46	1.44	1.33
r^2	0.9561 (0.9724)*	0.9851	0.9858	0.9832	0.9836	0.9864	0.9829	0.9747	0.9799	0.9646
		Downward curve (s^{-1})								
		40	35	30	25	20	18	15	10	
A	0.028 (0.026)*	0.031	0.031	0.031	0.032	0.033	0.034	0.034	0.034	
B	–	−0.16	−0.27	−0.41	−0.55	−0.82	−0.88	−0.91	−0.51	
r^2	0.9561 (0.9724)*	0.9646	0.9855	0.9855	0.9849	0.9907	0.9935	0.9812	0.9812	

*$\eta_0 = 1.92$. See text for explanation.

Values inside the parentheses are referred to the use of the experimental viscosity values instead of η_{ref}, as obtained by VM-10AL. We indicated such viscosity values as η_0 (i.e. the viscosity of the supernatant after centrifugation of the whole blood and the viscosity of the supernatant saline after centrifugation of the last washed blood). In detail, for whole blood and RBCs resuspended, $\eta_0 = 1.92$ and $\eta_0 = 1.18$ were adopted, respectively.

The comparison between the Hct-viscosity trends, as obtained by the two instruments, shows that the responses are satisfactorily similar. This aspect leads us to deepen the application of VM-10AL viscometer in an *ex vivo* study, where whole blood viscosity values of hospitalized patients were determined by means of the two instruments. Figures 5 and 6 describe the linear correlation between viscometer and rheometer measurements at two different shear rates (upward step, 20 s^{-1}; downward step, 25 s^{-1}, respectively).

In Table 4, results from linear fits of data to Eq. (3) for viscosity measurements as obtained by the two different techniques were reported. Furthermore, looking at the data on upward–downward steps, we also see that the two instruments demonstrated similar trends with each other, with the best value of r^2 equal to 0.9263 for the downward step, shear rate 10 s^{-1}.

Table 3

Coefficients for describing the dependence of washed blood viscosity (η_{washed}), as obtained by both VM-10AL and AR500, with respect to Hct, according to Eq. (2)

	VM-10AL	AR500								
		Upward curve (s^{-1})								
		8	10	15	18	20	25	30	35	40
A	0.028 (0.028)*	0.044	0.040	0.038	0.039	0.037	0.036	0.033	0.032	0.030
B	–	3.72	3.40	2.64	2.24	2.21	1.98	2.017	1.870	1.855
r^2	0.9902 (0.9911)*	0.8994	0.9180	0.9560	0.9379	0.9756	0.9793	0.9597	0.9449	0.9600
		Downward curve (s^{-1})								
		40	35	30	25	20	18	15	10	
A	0.028 (0.028)*	0.036	0.037	0.038	0.040	0.041	0.041	0.043	0.045	
B	–	0.24	0.06	−0.14	−0.50	−0.84	−1.02	−1.36	−2.13	
r^2	0.9902 (0.9911)*	0.9690	0.9735	0.9902	0.9931	0.9903	0.9880	0.9846	0.9556	

*$\eta_0 = 1.18$.

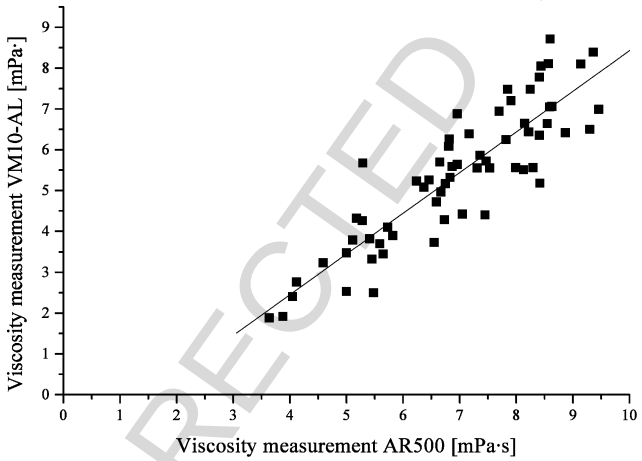

Fig. 5. Correlation between the viscosity data by VM-10AL and AR500 (upward step: 20 s^{-1}). The best-fit line was calculated using ordinary least squares regression, and the resulting line is shown in the graph.

4. Discussion

Differences between the two instruments in terms of blood viscosity determination *vs*. Hct may be attributable to several factors, first of all the mechanical solicitation in the case of AR500 rheometer. Assuming a disentanglement process would lead to a dependence of the viscosity due to adopted experimental protocol. Such a phenomenon is well evidenced at the level of the downward step, where the reversible RBC aggregation phenomenon disappears, leading to a broadening of the shear rate values and ultimately to a reduction of the Hct range within the selected shear rate limits [20]. The convergence to a Newtonian behaviour at high shear rates indicates the predominance of the oriented, deformed and disaggregated RBCs in the direction of the flow. At this situation, RBC roleaux includes not only the actual volume of the individual globule itself, but also the volume of plasma or saline immobilised within the closed aggregate [26].

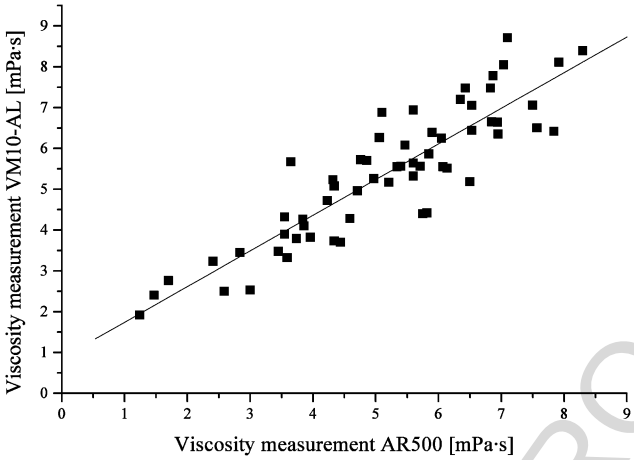

Fig. 6. Correlation between the viscosity data by VM-10AL and AR500 (downward step: 25 s^{-1}). The best-fit line was calculated using ordinary least squares regression, and the resulting line is shown in the graph.

Table 4
Fit parameters and coefficients of determination for viscosity measurements

Upward curve			
Shear rate (s^{-1})	m	q	r^2
8	0.479	0.298	0.7329
10	0.596	−0.377	0.8374
15	0.823	−1.043	0.8247
18	0.873	−0.979	0.8571
20	0.996	−1.533	0.8793
25	1.118	−1.715	0.8619
Downward curve			
Shear rate (s^{-1})	m	q	r^2
25	0.873	1.094	0.8904
20	0.819	0.920	0.8865
18	0.811	1.116	0.8910
15	0.755	1.338	0.8795
10	0.615	1.960	0.9263

As far as clinical sites are concerned, it is important that values obtained by new methods to be close to the target values. Although methods based on different operative principles produced different values, our comparison of *ex vivo* blood viscosity values shows that reasonably good agreement exists between the two techniques, resulting in linear coefficients of determination better than 0.73 (see Table 4). In a similar manner, the variability of the q terms may suggest that part of the variations associated with the two types of measurements could be related to the rheological behaviour under different shear–stress conditions for what AR500 determinations are concerned.

In conclusion, this paper reports comparison measurements of the viscosity of blood samples using two different techniques, with the aim to determine whether we were able to obtain comparable viscosity values. The viscometer based on piezoelectric torsional oscillation technology appears to be a suitable clinical measurement method able to overlay the limitations of the existing techniques, like complex-

ity and skilful operator necessity. In terms of standardization for hemorheological measurement, the accuracy criterion based on appropriate reference methods is under development.

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