AN IMPROVED METHOD FOR MEASURING RED BLOOD CELL FILTERABILITY

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The blood flow rate is, according to the Poiseuille law, a function of vessel size and fluid viscosity[3]. Viscosity is determined by a friction between adjacent layers of the fluid and, with vasoconstriction, it determines resistance to flow. Some fluids like water or plasma are called 'Newtonian' because they have a constant viscosity dependent only on temperature. Blood viscosity, on the contrary, is a function of the shear rate and is considered a 'non-Newtonian' parameter. For instance, this means that blood in veins (where shear rates are low) is more viscid than in arteries (where shear rates are high)[6,8].

The viscosity of whole blood mainly depends on the number and properties of red blood cells (RBC), i.e. the packed cell volume (PCV) and red cell deformability (RCD)[1]. Under normal conditions, leukocytes and platelets have little or no influence on blood viscosity. Of the plasma proteins, only fibrinogen is important, since at low shear rates it is able to produce RBC aggregation ('rouleaux')[5,10,15]. Among the numerous available methods for measuring RCD[2,4,11,12,14], the most widely used are those which measure the red cell filterability (RCF) through micropore membranes.

There are several methods for measuring RCF using whole blood or red blood cells suspended in saline solution or filtered plasma. All these methods are based on the same principle, differing only in the final stage. The end-points can be either the quantity of RBC filtered within a fixed time under variable or constant hydrostatic pressure[7,13] or the pressure increase under constant blood flow[9].

Key-words: Blood viscosity; Hemorheology; Red blood cell deformability; Red blood cell filterability.

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Since these methods are not well reproducible, employing a variation coefficient of 15-20% we have developed a new simple method able to measure RCF by weighing the amount of RBC filtered through micropore membranes (red cell filterability by weighing: RCFW). The results obtained with this method have been compared with those obtained using a widely employed method (Dormandy technique)[1].

MATERIALS AND METHODS

Materials

Phosphate-buffered saline (PBS): 39 g $Na_2HPO_4 \cdot 12\ H_2O$ and 3 g K_2HPO_4 are made up to 1 l with distilled water (pH 7.4, osmolality 300 mOsm/kg);

EDTA: 6 g of EDTA are dissolved in 10 ml of distilled water; 10 µl of this solution are used in order to prevent coagulation of 5 ml of blood;

glutaraldehyde: 0.5% in distilled water;

polycarbonate membranes (Nuclepore, batch no. 54FOC34) 13 mm in diameter with 5-µ pores.

Methods

RCFW - 5 ml of blood are drawn with minimal venous stasis and anticoagulated with EDTA. Blood is immediately centrifuged at 100 *g* for 3 min and then at 1,000 *g* for 7 min in order to obtain a better separation of red blood cells from the buffy-coat. Plasma and buffy-coat are aspirated and discarded. The packed RBC are then brought to the original volume with PBS. This procedure is repeated three times. More than 95% of leukocytes and platelets are eliminated by three washings. A suspension of 5% RBC in PBS is then prepared and 2 ml of this suspension are filtered for 30 sec, under gravity, through the micropore membranes and, finally, the filtrate is weighed.

In order to eliminate the effects of membrane variability, 2 ml of PBS are filtered through the same membrane for 30 sec before the RBC suspension, and then weighed.

The filterability index (FI) is calculated as follows:

$$FI = \frac{\text{weight of RBC filtrate}}{\text{weight of PBS filtrate}}$$

The procedure is carried out in triplicate.

Dormandy technique - Blood samples are anticoagulated with heparin and centrifuged at 1,000 *g* for 15 min; the plasma fraction is filtered in order to eliminate leukocytes and platelets. The buffy-coat is discarded and the RBC are resuspended in their own plasma at a 5% concentration. The FI is calculated by photometrically measuring the hemoglobin concentration of the RBC filtered during 1 min. The procedure is carried out in triplicate.

RESULTS

Reproducibility - The within-assay reproducibility was expressed as CV% and assessed for the two techniques from 10 replicates from a single blood donor.

method	normal range (mean ± 2SD)	within-assay precision			
		no.	mean	SD	CV%
RCFW	0.64 ± 0.12 (no. = 30)	10	0.68	0.04	5.8
Dormandy technique	0.80 ± 0.18 (no. = 20)	10	0.66	0.05	7.2

Tab. 1 - Comparison of the results obtained with the two methods.

Table 1 shows the results: CV% were 5.8 and 7.2 for RCFW and Dormandy technique, respectively.

Normal range - Normal ranges were established from 20 samples from normal subjects with Dormandy technique and from 30 samples from normal subjects with the RCFW. The FI value was 0.68 ± 0.12 (mean ± 2SD) and 0.80 ± 0.18 with RCFW and Dormandy technique, respectively.

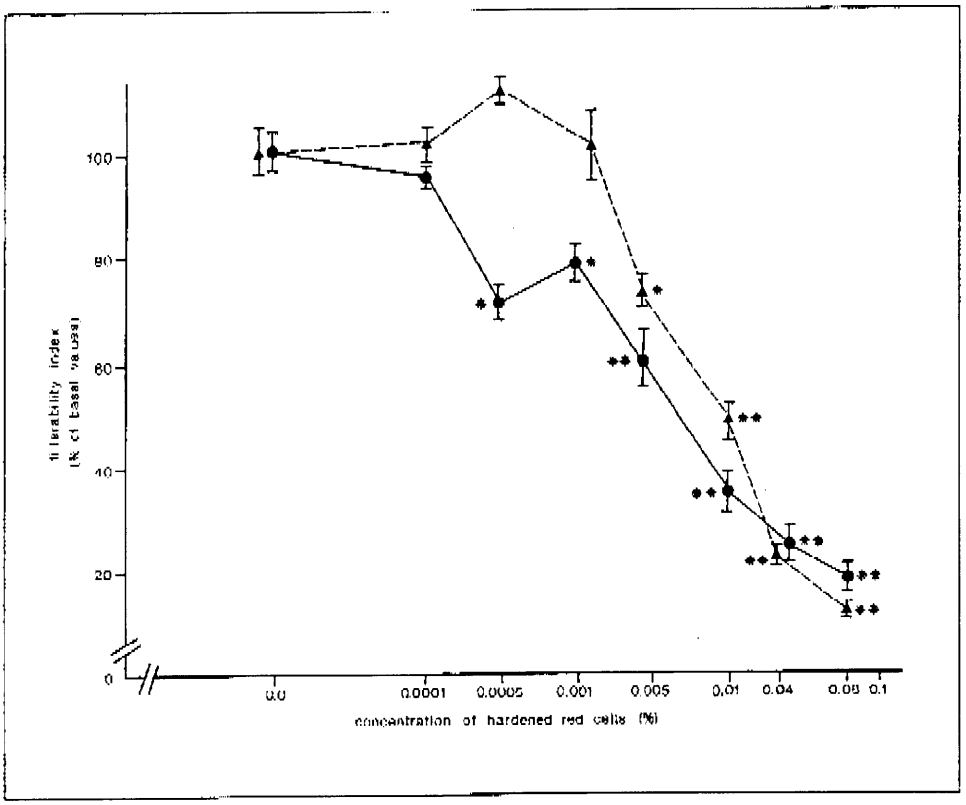
Sensitivity to hardened RBC - A 5% RBC suspension in PBS was rigidified with 0.5% glutaraldehyde at room temperature for 2h by continuous stirring. Increasing amounts of this suspension were added to samples from a single donor, in order to obtain hardened RBC concentrations ranging from 0.0001 to 0.08%. The suspensions were then processed by the two techniques. For a direct comparison of the two techniques, FI values obtained with increasing hardened RBC concentrations were expressed as percentages of the values obtained with both techniques when no hardened RBC have been added. With both techniques, FI values decrease with increasing concentrations of hardened RBC. However, with RCFW results significantly differed from normal RBC suspensions using concentrations as small as 0.0005%, while with Dormandy technique they significantly differed only at a concentration of 0.005%. In addition, the FI values obtained by RCFW were lower than those obtained by Dormandy technique at concentrations of hardened RBC up to 0.01% (fig. 1).

DISCUSSION

Little is still known about the relationship between RCD, RCF, blood viscosity and blood flow, but the results of some hemorheological investigations[2,9,13] suggest that filtration represents a good parameter for measuring RCD *in vivo*.

Since reproducibility is one of the major prerequisites for every clinically used technique, we have tried to improve it by modifying the end-point of currently used filtration techniques through micropore membranes essentially by weighing the amount of filtered RBC, related to the quantity of PBS filtered during the same interval of time, in order to compensate for small differences in the number and size of micropores of the different filters.

The method has proved to be reliable, with a reproducibility better than that of the widely used Dormandy technique. Moreover, its sensitivity to artificially hardened RBC seems to be greater and it is able to differentiate



* $p < 0.005$; ** $p < 0.001$ (*vs.* basal values).

Fig. 1 - Filterability index values obtained using the RCFW method (●——●) and the Dormandy technique (▲– – –▲) as a function of the hardened RBC concentrations in the filtered suspension. Results are expressed as percentages of the values obtained with both techniques when no hardened RBC have been added.

samples containing as little as 0.0005% hardened RBC, while the Dormandy technique does not differentiate them until a 0.005% concentration.

Since the filtration steps are similar for both techniques, the most likely explanation for the differences in performance is that variability in the photometric measurement of hemoglobin content is greater than that of direct weighing, making our modification of the technique more reproducible.

SUMMARY

A new simple method for measuring red blood cell (RBC) filterability by weighing the amount of RBC filtered through micropore membranes is described. Red cell filterability by weighing (RCFW) is determined on washed RBC resuspended in phosphate-buffered saline (PBS) at low (5%) hematocrit. The filtration step is performed using gravity alone and the amount of the filtered suspension is referred to the filtration of PBS alone through the same membrane. The new method shows a reproducibility better than that of the widely used Dormandy technique, with a lower coefficient of variation (5.8 *vs.* 7.2%) and a higher sensitivity to artificially hardened RBC.

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