An assessment of red cell deformability using a simple filtration method


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SUMMARY We assessed the simple method of measuring red cell deformability described by Reid et al. The technique was found to be reproducible. The validity of the method as a measure of red cell deformation was confirmed by (a) marked reduction of the deformability index after fixation of red cells with glutaraldehyde, and (b) an inverse correlation of deformability index with high-shear blood viscosity (r = 0.4; p < 0.001). There was no correlation of deformability index with low-shear blood viscosity, plasma viscosity, fibrinogen, or the white cell count. In normal subjects, deformability index was similar in males and females, and in smokers and non-smokers. Patients with acute myocardial infarction, or intermittent claudication, had reduced deformability compared to controls (p < 0.01).

Since it was first shown that red blood cells are deformed during their passage through capillaries, many methods for the measurement of red cell deformability have been described. There is, however, a need for a rapid, simple, and reproducible method of measuring red cell deformability suitable for large clinical studies. Reid et al. described such a simple method, recording the filtration time taken for 1 ml whole blood to pass through 5 μm polycarbonate filters under negative pressure, with correction for haematocrit. We have assessed the reproducibility of this method and investigated its relationship to changes in red cell deformability produced by glutaraldehyde fixation of red cells, or by deforming whole blood at high shear rates in a rotational viscometer. In view of the reported association between plasma fibrinogen and red cell deformability, as measured by a centrifuge method, we compared deformability with fibrinogen levels and with blood viscosity at low shear rates, which is highly correlated with red cell aggregation by fibrinogen. As white blood cells may be trapped in the filter pores, and as plasma viscosity may also affect the passage of red cells through the filter, we also correlated deformability with the white cell count and with plasma viscosity. We investigated the effect of sex and smoking habit on red cell deformability in a normal population and measured red cell deformability in patients with acute myocardial infarction and in patients with chronic peripheral arterial disease.

METHODS

Venous blood was anticoagulated with EDTA (1 mg/ml) for measurement of red cell deformability, haematocrit, blood viscosity, plasma viscosity, and white cell count.

RED CELL DEFORMABILITY

A detailed account of the basic method has already been published. The method was modified by filling the syringe beyond the 1 ml mark before applying negative pressure, and thus a constant speed was obtained before timing started. This improved reproducibility. The time taken for 1 ml of blood to flow was recorded, from which the volume of blood filtered per minute was calculated. Multiplication of this value by the haematocrit value gave the volume of red cells filtered per minute (red cell deformability index; RCD). The filters were supplied by Nucleopore, and as a large variation was found between batches, the same batch number was used throughout.

FIBRINOGEN LEVELS

These were measured in citrated plasma by a thrombin-time method, using a Dade Fibrometer and standards.
HAEMATOCRIT

Haematocrit measurements were made with a micro-
haematocrit centrifuge (Hawksley), spinning at
13 000 g for 5 minutes. Plasma viscosity was measured
at 37°C in a BS-M3 capillary viscometer. Blood viscos-
ity was measured at 37°C in a Contraves
LS-30 rotational viscometer at a low shear rate
(0.94 s⁻¹) and a high shear rate (94.5 s⁻¹). Viscosity
was corrected to a standard haematocrit of 0.45
using a regression equation obtained from the
population studied. White cell count was measured
by Coulter 'S'.

Samples were taken from healthy hospital staff
and from patients in medical and surgical wards.
Patients with acute myocardial infarction were
studied within seven days of onset of an acute
transmural infarction with typical history and serial
changes in electrocardiograms and serum enzymes.
Patients with chronic peripheral arterial disease had
a typical history of intermittent claudication and
absent foot pulses.

Differences in means of the variables studied were
analysed by Student's t test, and correlations were
determined by the method of least squares.

Results

RELATION TO HAEMATOCRIT

Figure 1 shows the correlation between whole blood
filtration rate and haematocrit in 200 subjects.
Reconstitution experiments to give haematocrit
values in the range 0.30-0.60 resulted in a significant
negative correlation (r = 0.99, p < 0.05) between
whole blood filtration rate and haematocrit. Multi-
plication by the haematocrit to obtain the RCD
abolished this correlation (r = 0.01).

REPRODUCIBILITY

On each of 20 normal samples, seven measure-
ments of RCD were performed; the mean coefficient
of variation was found to be 3.7%. We therefore
decided to take the mean of triplicate measurements
on each sample.

EFFECT OF FIXATION OF RED CELLS BY
GLUTARALDEHYDE

In order to validate the theory that rigid cells would
be detected by the method, red cells were treated
with 1.5% (v/v) glutaraldehyde in phosphate buffered
saline at 4°C for 48 hours, as described by Largo
et al.5 The volume of red cells was 0.53 ml/min before
fixation and 0.008 ml/min after treatment, indicating
that the rigid cells were much less able to pass through
the pores in the filter. A control experiment storing cells at 4°C for 48 hours
in phosphate buffered saline without glutaraldehyde
produced a less marked loss of deformability
(0.50 ml/min before, and 0.28 ml/min after,
treatment).

CORRELATION WITH HIGH SHEAR BLOOD
VISCOSITY

In a population of 200 normals and patients, RCD
was correlated inversely with blood viscosity at the
high shear rate of 94.5 s⁻¹, corrected to a standard
haematocrit of 0.45 (Fig. 2).
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Red cell deformability in relation to sex and smoking

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 23</td>
<td>N = 15</td>
<td>N = 38</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>Age = 31.2±8.1</td>
<td>Age = 26.9±6.4</td>
<td>Age = 29.5±7.7</td>
</tr>
<tr>
<td></td>
<td>RCD = 0.49±0.06</td>
<td>RCD = 0.52±0.05</td>
<td>RCD = 0.50±0.06</td>
</tr>
<tr>
<td>Smokers</td>
<td>N = 15</td>
<td>N = 13</td>
<td>N = 28</td>
</tr>
<tr>
<td></td>
<td>Age = 27.5±8.5</td>
<td>Age = 29.2±10.2</td>
<td>Age = 28.3±9.2</td>
</tr>
<tr>
<td></td>
<td>RCD = 0.48±0.08</td>
<td>RCD = 0.48±0.06</td>
<td>RCD = 0.48±0.07</td>
</tr>
<tr>
<td>Total</td>
<td>N = 38</td>
<td>N = 28</td>
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<td>Age = 29.3±8.3</td>
<td>Age = 28.1±8.2</td>
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</tr>
<tr>
<td></td>
<td>RCD = 0.48±0.07</td>
<td>RCD = 0.50±0.05</td>
<td>RCD = 0.49±0.06</td>
</tr>
</tbody>
</table>

Correlation with other blood variables

Within the same population as above, it was found that there were no significant correlations between RCD and plasma viscosity (r = 0.05), low shear blood viscosity (r = 0.017), fibrinogen (r = 0.010), or the white cell count (r = 0.17).

Effect of sex and smoking

A group of 60 normals less than 50 years old were subdivided for sex and smoking habit as shown in the Table. No significant difference was found between males and females, or between smokers and non-smokers (P > 0.05).

Relation to vascular disease

Thirty men admitted to hospital suffering from acute myocardial infarction or intermittent claudication were studied and compared with a group of age-matched controls. Figure 3 shows that these patients suffering from acute and chronic vascular disease had significantly more 'rigid' cells (p < 0.01) than the control group.

Discussion

We have found the filtration method of Reid et al. to be a reproducible and relatively simple means of estimating red cell deformability, allowing large numbers of patients to be studied. We have confirmed that the time taken for whole blood to filter is strongly dependent on the haematocrit, and that simple multiplication of the volume of blood filtered per minute by the haematocrit abolishes this correlation. We investigated the validity of the method as a measure of deformability in two ways. Firstly, fixation of red cells by glutaraldehyde produced a marked fall in the deformability index, as shown previously by Schmid-Schönbein et al. Secondly, the deformability index correlated with blood viscosity measured at a high shear rate. Schmid-Schönbein et al. and Chien have shown that blood viscosity falls at high shear rates, due to increased deformation of red cells. Our findings, therefore, suggest that results using the method of Reid et al. are indeed related to red cell deformation.

We found no correlation between red cell deformability and plasma fibrinogen, or between red cell deformability and blood viscosity at low shear rates. It is, therefore, unlikely that the filtration method measures red cell aggregation. Neither did we find a correlation between red cell deformability and plasma viscosity, or between red cell deformability and the white cell count up to 15 x 10⁹/l. We therefore doubt whether plasma viscosity effects, or blockage of pores by white cells, have major effects on red cell deformability values. These findings suggest that it is valid to measure filtration rates of whole blood samples without removal of white cells or resuspension of red cells in solutions other than plasma. However, the method may not be applicable to samples with very high white cell

![Figure 3: Red cell deformability in myocardial infarction and intermittent claudication.](http://jcp.bmj.com/)

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**Fig. 3** Red cell deformability in myocardial infarction and intermittent claudication.
counts (leukaemia) or very high plasma viscosity (macroglobulinaemia or myeloma). We found no relation of red cell deformability to sex or to smoking habit.

It has been suggested that red cells which have impaired deformability may impair blood flow in the microcirculation, the cells being less able to squeeze through capillaries. It is therefore of interest to study red cell deformability in patients with vascular disease. Our finding that patients with chronic peripheral arterial disease had less deformable cells agrees with the study of Reid et al. We have also shown that men with acute myocardial infarction have fewer deformable cells. It therefore appears that the method described by Reid et al. will be useful in the study of red cell deformability in vascular disease.

References

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