Effect of reconstructive vascular surgery on red cell deformability—preliminary results

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SUMMARY Using a simple filtration method, red cell deformability was measured in healthy control subjects and in patients with peripheral vascular disease. Impaired red cell deformability was demonstrated in patients with rest pain or gangrene and in patients with intermittent claudication. An improvement in red cell deformability was demonstrated after successful reconstructive vascular surgery in both patient groups. An improvement in red cell deformability was demonstrated in patients undergoing major limb amputation.

Red cell deformability (RCD) has been defined as the geometric and physical characteristics which permit a red cell, whose greatest diameter may exceed $8\text{ }\mu\text{m}$ to pass through normal capillaries, of diameter $3\text{--}12\text{ }\mu\text{m}$.¹ The ability of normal red blood cells to deform is important, both in the circulation through the microvasculature and, at high shear rates, in the production of smooth laminar flow in large vessels.² Impaired RCD has been demonstrated in peripheral vascular disease³⁻⁵ and may reflect the severity of disease.⁴ Further, as RCD is an important determinant of whole blood viscosity and hence blood flow, impaired RCD may exacerbate states of peripheral vascular insufficiency.⁷

Those mechanisms controlling RCD are still subject to considerable speculation and little information is available on the changes in RCD after surgery on the peripheral vascular tree. This study examines the changes in RCD after aorto-iliac and femoropopliteal reconstruction of the peripheral vascular tree.

Patients and methods

Thirty-three unselected, non-diabetic patients were studied. Red cell deformability was measured in these patients and in a further 23 healthy control subjects. Informed consent was obtained. After clinical examination, ankle pressure indices were measured using a Doppler probe (10 mHz). Patients with intermittent claudication had their claudication

distance measured, using a treadmill at 3 km/h on a 10% incline. Venous blood was withdrawn from an antecubital vein, without stasis, for routine full blood count, blood sugar, blood lipids, plasma protein electrophoresis and coagulation profile; further 20 ml was anticoagulated with 30 mg of ethylenediamine tetra-acetic acid (EDTA) for measurement of RCD and plasma viscosity.

SURGERY

Twenty-five patients underwent reconstructive vascular surgery (13 for intermittent claudication and 12 for severe ischaemia producing rest pain or gangrene). Eight patients had major limb amputations, of whom two died in the immediate post-operative period. Two weeks after surgery, each of the 31 patients available for study was reassessed by clinical examination, measurement of their ankle pressure indices and claudication distances, where appropriate. Measurement of the haematological and haemorheological parameters was repeated. No patients were receiving drugs known to alter RCD at the time of follow-up, though 12 patients were receiving Dextran 70 at the time the initial (preoperative) blood samples were taken. Nine patients had aorto-bifemoral grafts and all received either 2 (4 patients) or 3 (5 patients) units of donor blood. A further 9 patients underwent femoropopliteal bypass, one of whom received a donation of 2 units of blood. In the remaining 15 operations (8 amputations, 3 aorto-iliac endarterectomies, 1 femoro-femoral cross-over, 1 EDFA and 2 ilio-femoral bypass) no blood was transfused.

In 12 patients of comparable age and sex (9 male, 3 female, aged 47–76 yr, mean age 60) undergoing major surgery (herniorrhaphy and abdominal surgery), RCD was measured preoperatively and two weeks postoperatively. One of the control patients received one unit of blood during surgery, no other control patients had blood transfusion. Since stored blood is known to show deteriorating red cell filtrability with time, the transfusion of up to 3 units in 10 of the 31 patients surviving surgery is, if anything, likely to produce impairment of red cell deformability in the study group as compared with the controls.

MEASUREMENT OF RED CELL DEFORMABILITY

RCD was measured using a modification of Buchan's technique.⁸ EDTA anticoagulated blood was centrifuged at 2,500 rpm for 15 min and the plasma aspirated. A sample of concentrated erythrocytes, virtually free of leucocytes, was obtained by passing the tip of a Pasteur pipette through the buffy layer and withdrawing as much as possible of the red cell mass from the bottom of the sample. This was found to result in less leucocyte contamination than removing and discarding the buffy layer. The erythrocytes were resuspended in their own plasma and the haematocrit adjusted to 0.300 (± 0.016). Background leucocyte and platelet counts were checked on a Coulter Model S Plus, to ensure adequate removal of these cells. RCD was measured using Nucleopore polycarbonate membranes of 5 μ m pore diameter,⁹ all the membranes being from the one batch (Fig. 1). Filters were assembled in "Pop-Top" filter holders (Nucleopore) and, after assembly the dead space above the membrane was filled with the red cell suspension. The barrel of 1 ml syringe was fitted to the holder and then filled using a Pasteur pipette, to above the 1 ml mark. A suction pressure of 20 cm of water was applied across the filter and the time taken for 1 ml of blood to pass through the filter was recorded. Each sample was estimated in quadruplicate and the results expressed

as a mean, in seconds/ml. All measurements were carried out within two hours of venesection. Plasma viscosity was measured using a Coulter Harkness Viscometer (Coulter Electronics, Luton, Beds) at a temperature of 25°C.

The results did not show a "normal" distribution and therefore non-parametric analyses were applied, the Mann-Whitney U test being used to compare control subjects with each of the patient groups and the Wilcoxon signed-rank test to examine the change in RCD and plasma viscosity after surgery.

Fig. 2 Red cell deformability preoperatively.

Results

Thirty-three patients, 26 men and 7 women, mean age 62 (range 45–83 yr) were studied. Thirteen patients had intermittent claudication (claudicant group) and 20 had rest pain or gangrene (ischaemic group). Twenty-three control subjects were also studied. These were healthy members of the medical, laboratory and nursing staff. The mean RCD ($\pm$ SD) in the control subjects and each of the patient groups is shown in Fig. 2. Deformability in each of the two patient groups was significantly worse than in the control group ($p = 0.001$). While the mean values in the claudicant and ischaemic groups differed, this did not reach statistical significance, using a non-parametric analysis ($p = 0.06$).

Table 1 Change in red cell deformability after surgery

Group	n	Preop RCD	SD	Postop RCD	SD	p
Claudication	13	35.5	7.5	31.7	7.2	0.02
Ischaemia	12	39.8	7.9	31.7	6.3	0.01
Amputation	6	44.2	12.5	31.1	8.8	0.03
Overall	31	38.9	9.1	31.6	6.9	<0.001

Fig. 1 Apparatus for measurement of red cell deformability.

Table 2 *Change in ankle pressure index after reconstructive vascular surgery*

Group	n	Preop API	SD	Postop API	SD	p
Claudication	13	0.49	0.2	0.75	0.14	<0.001
Ischaemia	12	0.15	0.19	0.47	0.19	<0.001
Overall	25	0.33	0.26	0.62	0.22	<0.001

The RCD in the 23 control subjects was 22.8 (SD 3.2). In the 31 patients examined two weeks after surgery mean RCD improved significantly from 38.9 s/ml to 31.6 s/ml ($p < 0.001$) but remained significantly different from the control range ($p < 0.001$) (Table 1). Within each of the patient groups, RCD improved significantly (Fig. 3). It can be seen from Table 2 that this was accompanied by an improvement in ankle pressure index (API) and claudication distance, where applicable. It is interesting to note that the post-operative RCD in each of the groups was of the order of 31 s/ml. Plasma viscosity was abnormal in the patients with rest pain or gangrene (1.83 mPa.s) but did not change significantly after surgery, including amputation. In the claudicant group, preoperative plasma viscosity was normal (1.71 mPa.s) and it deteriorated postoperatively (1.83 mPa.s). This deterioration in plasma viscosity in the claudicant group was statistically significant ($p = 0.02$), and is not explicable. In the 12 patients undergoing operation in the control group, there was no significant change in RCD or plasma viscosity postoperatively.

Discussion

Abnormal RCD has been demonstrated in peripheral vascular disease by a number of workers,³⁻⁵ who have shown significant differences between control subjects, patients with intermittent claudication and patients with severe ischaemia.⁴ However,

early workers failed to remove the leucocytes prior to filtration, a factor that may account for their result, since leucocytes have been shown to prolong the time taken for passage of erythrocytes through the Nucleopore membrane,⁸ and the leucocyte count may be raised in severe peripheral vascular insufficiency. We feel the background leucocyte count prior to filtration should be $0.5 \times 10^9/l$ and preferably $0.3 \times 10^9/l$. Although our results demonstrate a difference in the mean RCD in patients with intermittent claudication and those with severe ischaemia, this does not reach formal statistical significance ($p = 0.06$).

The mechanism by which this abnormal deformability is produced remains unresolved. There is evidence in support of "plasma factor" producing abnormal deformability in Raynaud's syndrome⁶ and it seems likely that there is a similar factor in peripheral vascular disease, since, when blood is filtered in saline rather than native plasma, RCD is not significantly different in patients and controls (unpublished observations).

Matthews has shown a deterioration in a number of haemorrhological factors, notably RCD, in the 24 h after major surgery, including reconstructive vascular surgery.¹⁰ However, he is undoubtedly measuring the acute effects of the surgical trauma. It seems logical that relieving peripheral vascular insufficiency will improve RCD. As far as we are aware, the longer term effects of reconstructive vascular surgery on deformability have not been examined. A longer term study is being undertaken in this department and the results will be published elsewhere. There seems no clear cut relation between plasma viscosity and RCD demonstrated by these results.

Ideally, it would be best to compare the changes in RCD after reconstructive vascular surgery with an age- and sex-matched group of patients undergoing comparable surgery. In the authors' opinion, it is not possible to compare any operative procedure with

Fig. 3 *Effect of surgery on red cell deformability.*

salvage surgery for incipient gangrene. The controls were chosen because the operative procedures that they were undergoing were of comparable "severity" and blood loss would be similar. In fact, some of the vascular patients required blood transfusion which may have produced a temporary deterioration in RCD post-transfusion.

Four patients showed a deterioration in RCD after surgery in the present study. On postoperative assessment, one patient was severely limited by contralateral claudication and one by severe angina (which may explain these results). The remaining two patients appeared to have had a successful outcome, yet showed a slight deterioration in RCD postoperatively. These latter results cannot be explained.

There appeared to be no difference in the change in RCD in men and women patients, nor was there any significant difference when a prosthetic graft was used. Patients undergoing aorto-iliac surgery showed a slightly greater improvement in RCD, though this was not statistically significant and was probably due to revascularisation of the contralateral limb.

Conclusions

These findings confirm that RCD is abnormal in peripheral vascular disease. After reconstructive vascular surgery there was a significant improvement in RCD, unrelated to changes in other haemorrhological factors. Furthermore, an improvement in RCD was noted after major limb amputation. It appears that the abnormal RCD in peripheral vascular disease is secondary to the impaired circulation and is rapidly reversible, but the mechanism by which this is mediated remain uncertain.

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References

- ¹ Weed RI. The importance of erythrocyte deformability *Am J Med* (Editorial) 1970;**49** (2):146–50.
- ² Schmid-Schonbein H. Critical closing pressure of yield shear stress as a cause of disturbed peripheral circulation? *Acta Chirurg Scand* 1976;suppl no 465:10.
- ³ Dormandy JA, Flute PT, Pymak-Micholirski SM, Reid HL. Erythrocyte deformability and blood viscosity in peripheral circulatory disease. *J Physiol* 1977;**275**:19P–20P.
- ⁴ Reid HL, Dormandy JA, Barnes AJ, Lock PJ, Dormandy TL. Impaired red cell deformability in peripheral vascular disease. *Lancet* 1976;i:666–8.
- ⁵ Ehrly AM, Kohler MJ. Altered deformability of erythrocytes from patients with chronic occlusive arterial disease. *Vasa* 1976;**5** (4):319–22.
- ⁶ Dodds AJ, O'Reilly MJG, Yates CJP, Cotton LT, Flute PT, Dormandy JA. Haemorrhological response to plasma exchange in Raynaud's syndrome. *Br Med J* 1979;ii:1186–7.
- ⁷ Leading article. Red cell deformability. *Lancet* 1978;ii:1348–9.
- ⁸ Buchan PC. Evaluation and modification of whole blood filtration in the measurement of erythrocyte deformability in pregnancy and the newborn. *Br J Haematol* 1980;**45**:97–105.
- ⁹ Reid ML, Barnes AJ, Lock PJ, Dormandy JA, Dormandy TL. A simple method for measuring erythrocyte deformability. *J Clin Pathol* 1976;**29**:855–8.
- ¹⁰ Matthews PN. In: *Clinical aspects of blood viscosity and cell deformability*. Berlin, Heidelberg, New York: Springer-Verlag, 1981.

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