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Red cell deformability in patients with chronic atheromatous ischemia of the legs

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SUMMARY

Background: *It has been shown that atherosclerosis can cause not only lowering of the perfusion pressure gradient at the macro/micro circulation level, but can bring about untoward rheological changes such as e.g. loss of the red blood cells (RBC) elasticity. This can participitate in mechanism of impairment of the blood flow through the microcirculation as well. The aim of this study was to measure RBC elasticity in the claudicants, and to evaluate the effect of the applied treatment either conservative or surgical revascularisation on this rheological parameter.*

Material and methods: *RBC deformability was examined in claudicants 83 of them (mean claudication distance 400 m) were treated conservatively (walking exercise on a treadmill and Pentoxyphilline 600 mg b.i.d.) 44 claudicants with the critical leg ischemia (mean claudication dostance below 50 m or rest pain) underwent surgical revascularisation. The measuremants of RBC elongation (under selected values of the shear stress) were made on entry to the study, after 6 weeks, and 12 weeks of observation.*

Results: *After 12 weeks of conservative treatment, RBC elasticity both at rest and after walking exercise significantly increased. In the patients undergoing surgery mean RBC elasticity both at rest and after walking exercise 12 weeks following surgery was not significantly different compare with the respective levels at the beginning of the study.*

Conclusions: *In the group of claudicants with less advanced atherosclerosis (conservative group), the treatment brought about significant increase of RBC elasticity. On the contrary in case of the patients with advanced atherosclerosis and critical leg ischemia revascularisation alone appeared to be ineffective in correction of untoward rheologic alteration such as rigidity of the erythrocytes.*

BACKGROUND

Studies on microcirculation have shown that blood circulates through the capillaries according to the gradient of perfusion pressure between the arterial and venous ends of the circulation. The blood flow necessary to maintain a normal rate of metabolism in the surrounding tissues which enables adequate exchange between blood and extravascular space is subject to complex autoregulation by the myogenic and metabolic mechanisms, and by the so-called 'vasomotion'. The myogenic mechanism regulates the diameter of the vessels in the microcirculation unit, depending on the gradient of perfusion pressure. The mechanism of metabolic regulation mediated by adenosine is triggered by changes in partial oxygen pressure in tissues. The concept of vasomotion is a quite recent development; it refers to the transient capability of vessels to extend and shrink in vibrating movements. These rhythmic changes in the vessels' caliber facilitate the redistribution of circulating blood cell mass in the capillaries, as well as changes in the blood concentration in the capillaries, with the immediate effect on the oncotic-to-hydrostatic pressure ratio [1,2].

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One of the most common causes of insufficient blood supply in peripheral tissues is atherosclerosis. The pathomechanism of atheromatosis, besides a lowered perfusion pressure gradient and limited tissue supply, includes equally important alterations in blood rheology. Patients with atheromatosis of the lower extremities have been reported to develop high levels of hematocrit, high fibrinogen concentration, increased plasma and blood viscosity, and rigidity of blood morphotic components, both red and white cells. These pathologically altered rheologic properties are capable of impairing blood flow through microcirculation when the perfusion pressure level is lowered [2–4].

The ability of red blood cells (RBCs) to deform is of vital importance, because it enables them to change their shape and traverse microcirculation and capillaries with a diameter smaller than their own. In response to shear stress, RBCs change their shape from biconcave disk to ellipsoid. They align themselves parallel to the flow direction, and their membranes may rotate around the fluid-like cytoplasm in the so-called 'tank-tread'-like motion [5–7]. Under normal circumstances these properties enable RBCs 7 μm in diameter to traverse capillaries with a diameter of 3–5 μm and supply oxygen to tissues.

The elasticity of RBCs has been demonstrated to affect their life span. In the spleen, RBCs squeeze through narrow slit-like sinuses, 0.5–1.0 μm in diameter. The spleen plays the role of a powerful filter, in which rigid erythocytes are sequestered and then destroyed.

Studies on the structure of erythrocytes have shown that the loss of elasticity and the ability to deform may be attributed to transformations caused by chemical substances, metabolic defects, hypoxia, and impaired capacity to regenerate energy [8–10].

Rigid RBCs have been identified in various diseases, including hereditary spherocytosis, anaemia (autoimmune hemolytic or sickle cell), metabolic disorders accompanying septic conditions, renal insufficiency, and diabetes. The loss of RBC elasticity has also been well documented in atheromatosis in the heart, brain, and lower extremities [11,12].

The aim of our study was to measure RBC elasticity in patients at different stages atheromatosis of the lower extremities, and to evaluate the outcomes of conservative treatment and surgical revascularization in respect to this rheological parameter.

MATERIAL AND METHODS

The study involved patients referred to the Peripheral Vascular Disease Outpatient Clinic in our Department with a diagnosis of chronic atheromatous ischemia of the leg. The patients were subjected to a non-invasive examination using a Doppler Blood Detector (Meda Sonics, USA) to monitor the flow signal in the dorsal artery or in the posterior tibial artery. In all cases, the systolic pressure in these arteries was measured, and the ankle/brachial index (A/BI) was also estimated on a treadmill (3 km/h, 10° elevation). The claudication distance was recorded, as well as the maximum walking time (MWT), the time after which the exercise was discontinued due to increasing pain. The post-exercise pressure drop in the dorsal or posterior tibial arteries was graphed in the form of curves, and the surface of the so-called 'ischemic field' was estimated. The ischemic field was integrated using WindCad software [13].

A total of 127 patients with obliterating atheromatosis were divided into two study groups. Group A consisted of 83 patients receiving conservative treatment, with a mean claudication distance of 400 m and an A/BI between 0.9–0.75. Group B consisted of 44 patients who underwent surgical revascularization, with a mean claudication distance below 50 m and an A/BI below 0.55, or with pain at rest. The control group included 22 age-matched non-claudicants admitted to our Department for elective surgery, most frequently laparoscopic cholecystectomy or hernioplasty. The clinical data of the patients included in the study are presented in Table 1.

Before entering the study all the patients were fully informed of the methods the be applied and the

Table 1. Clinical discription of the patients: controls (n=22), conservative menagement (n=83), surgical revascularisation (n=44).

	Groups					
	Control		Conservative		Surgical	
	M	F	M	F	M	F
Number	14	8	64	19	35	9
Mean age (years)	57 (34-78)		58 (35-74)		58 (45-73)	
Mean claudication time (years)	–		2.5		3.9	
Hypertension (%)	54		61		6.4	
Diabetes (%)	7		9		11	
Coronary insufficiency (%)	48		52		62	
Smoking (%)	66		75		84	

M - male; F - female

Table 2. Surgical revascularisation (n=44) types, number.

Type of operation		Number of patients
I. Aorto-famoral bypass	one side	6
	both side	14
II. Aorto-iliacal bypass	one side	3
	both side	4
III. Femoro-ppliteal bypass (supragenicularis)		5
IV. Femoro-popliteal bypass (infragenicularis)		12
Total number		44

aim of the study, and written consent was obtained from all of them.

Conservative treatment involved walking exercises on the treadmill and pharmacology. One walking exercise consisted of three walking units, each of them corresponding to 80% of the MWT calculated individually for each patient. Walking exercises supervised by a specially trained nurse and physician were continued three times a week for 12 weeks. Additionally, the patients received 600 mg Pentoxyphilline orally b.i.d. The patients qualified for surgical revascularization received vascular bypass prostheses (Dallon, Goretex) (see Table 2).

RBC deformability was measured using a Rheodyn SD Laser Shear Stress Diffractometer (Myrenne GmbH Germany) with the capacity to estimate RBC elasticity in a wide range of shear stress from 0.30 to 60.0 Pa. The data obtained were computer processed and deformability curves were generated.

RBC elasticity was assessed in both the conservative and surgical treatment groups, at rest and immediately after walking exercise (corresponding to three times the MWT of the individual patient), once at the beginning of the study, again after 6 weeks, and finally after 12 weeks. Venous blood samples were collected in plastic syringes containing heparin (10 IU/ml). 30 µl of blood were mixed with 2 ml/g of buffer solution prepared from 1 liter of physiologic NaCl solution (pH = 7.4, osmolarity 290–300 mOsm). 200 mg of Dextran was added to 800 ml of the solution prepared in this way. The viscosity of the solution was measured at a temperature of 23°C and corrected to 24 mPa by adding physiologic salt or Dextran.

These parameters were measured at the Department of Hematology and Experimental Toxicology at the Jagiellonian University's Institute of Zoology by Prof. Z. Dąbrowski.

Table 3. Hemodynamic parameters, Mean values, standard deviations.

	Initial values	After 6 weeks	After 12 weeks
Conservative treatment			
SP (mm Hg)	75.00±17.40	80.00±15.00	78.00±14.00
AB/I	0.71±0.04	0.68±0.03	0.72±0.09
CAT (min)	2.80±0.30	5.10±0.40	7.30±0.90*
MWT (min)	4.90±0.40	9.70±0.80*	11.80±1.70*
Ischemic window (mm^2)	1430.0±115.0	985.0±84.0*	698.0±66.0*
Surgical revascularisation			
SP (mm Hg)	38.00±14.00	105.0±12.0*	101.0±27.0*
AB/I	0.48±0.04	0.98±0.09*	0.94±0.07*
CAT (min)	1.40±0.50	> 10 min	> 10 min
MWT (min)	3.70±1.10	> 15 min	> 15 min
Ischemic window (mm^2)	1973.0±286.0	167.9±24.0**	186±18.0**

* statistically significant difference $p<0.01$ compare with the initial values
** statistically significant difference $p<0.001$ compare with the initial values
SP - systolic pressure; AB/I - ankle/brachial index; CAT - claudication appearing time; MWT - maximal walking time

Statistical analysis

The mean and standard deviations of the parameters were calculated, and statistical significance of the values obtained was estimated using the Student t-test (the significance level was set at $p<0.05$).

RESULTS

The patients undergoing both conservative and surgical treatment benefited from the therapy they received. This was indicated by prolonged MTWs, delayed claudication, and reduced ischemic fields. Moreover, in the surgical group an increase was observed in the peripheral systolic pressure and A/BI (cf. Table 3).

RBC deformability under the minimum shearing stress of 0.30 Pa and the maximum of 60Pa, estimated both at rest and after walking exercise, was significantly higher in the controls as compared to the conservative group ($p<0.05$), and to the surgical revascularization group ($p<0.001$) (cf. Table 4 and Fig. 1).

After 12 weeks of conservative treatment, the patients' RBC elasticity was significantly increased, both at rest and after walking exercise, especially at the lowest and highest levels of shear stress ($p<0.05$) (cf. Table 5 and Fig. 2). In the patients who underwent surgery, the mean parameters of RBC elasticity measured at rest and after exercise 12 weeks after surgery were not si-

Table 4. Mean elongation changes (%), w of the red cells (blood viscosity 24 mPa/sec.) in the groups: Control (n=22), Conservative (n=83), Surgical (n=44).

Shear stress [Pa]	Red cell elongation [%]					
	Control group		Conservative group		Surgical group	
	Resting values	Postexercise values	Resting values	Postexercise values	Resting values	Postexercise values
0.30	7.20	6.77	4.80	2.31	1.32	0.81
0.60	10.90	9.83	5.71	3.68	2.67	1.10
1.20	26.42	24.31	14.92	9.90	3.14	1.90
3.00	36.71	37.06	17.81	11.63	7.85	5.60
6.00	44.21	41.50	31.62	29.62	9.63	7.93
12.00	50.96	51.96	43.11	33.80	19.29	15.63
30.00	54.06	55.76	47.56	39.66	28.01	19.78
60.00	76.70	74.66	57.81	48.75	37.81	23.75

Table 5. Mean elongation changes (%), w of the red cells (blood viscosity 24 mPa/sec.) in the conservative groups: Initial, after 6 weeks and after 12 weeks values.

Shear stress [Pa]	Red cell elongation [%]					
	Initial values		After 6 weeks		After 12 weeks	
	Resting values	Postexercise values	Resting values	Postexercise values	Resting values	Postexercise values
0.30	4.80	2.31	4.709	2.81	8.93	6.63
0.60	5.71	3.68	8.11	7.43	10.57	8.87
1.20	14.92	9.90	17.62	15.61	19.34	15.71
3.00	17.81	11.63	24.13	20.18	25.08	22.68
6.00	31.62	29.64	33.07	31.19	30.02	29.16
12.00	43.11	33.80	41.91	39.00	49.03	39.81
30.00	47.56	39.66	63.43	46.00	61.74	50.61
60.00	57.81	48.75	70.63	61.73	72.03	68.78

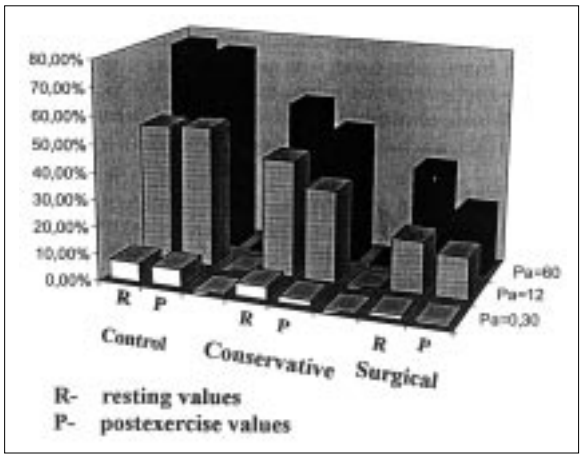

Figure 1. Resting and postexercise elongation changes [%] of the red cells (blood viscosity 24 mPa/sec.) in the groups: Control, conservative, surgical.

gnificantly different when compared with the respective levels at the beginning of the study (cf. Table 6 and Fig. 3).

DISCUSSION

It has been demonstrated in the literature that the structure of the erythrocyte membrane substantially influences its mechanical properties. Under the lipid shell there is a bilayer of proteins that determines the shape of the red cell. Hemoglobin, the main protein in the erythorcyte, transports oxygen and regulates intracellular viscosity, determining the rheologic properties. About 70% of the cell mass is water, the volume of which is regulated in the process of osmosis by the intracellular K^+ and extracellular Na^+ ions.

An erythrocyte is a flattened biconcave disk, the shape of which may easily be changed. The biochemical aspects of the membrane proteins that enable the erythrocyte to maintain its biconcave shape are not yet fully understood [6,7,14]. It has been shown that impaired energy metabolism has an impact on the mechanical properties of RBCs, and that maintaining the biconcave disk shape requires energy. A decreased level of ATP can produce as much as

Table 6. Mean elongation changes (%), w of the red cells (blood viscosity 24 mPa/sec.) in the surgical groups: Initial, after 6 weeks and after 12 weeks values.

Shear stress [Pa]	Red cell elongation [%]					
	Initial values		After 6 weeks		After 12 weeks	
	Resting values	Postexercise values	Resting values	Postexercise values	Resting values	Postexercise values
0.30	1.32	0.81	1.62	1.21	1.53	1.01
0.60	2.67	1.10	3.57	1.46	3.01	1.51
1.20	3.14	1.90	3.70	2.98	3.94	2.30
3.00	7.85	5.60	5.94	4.63	3.71	4.75
6.00	9.63	7.93	7.63	6.74	10.52	8.30
12.00	19.27	15.63	18.27	17.58	20.13	18.70
30.00	28.01	19.78	29.01	21.56	23.15	20.73
60.00	27.81	23.75	38.62	33.76	31.67	25.51

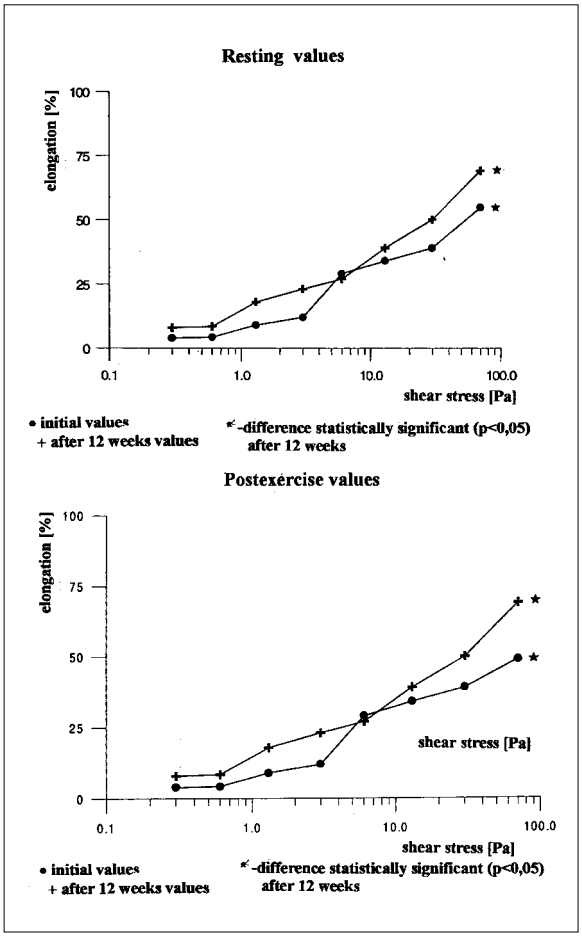

Figure 2. Comparison of the percentage elongation changes of the red cells: resting and postexercise, in the conservative group. Measured on entry and after 12 weeks of treatment.

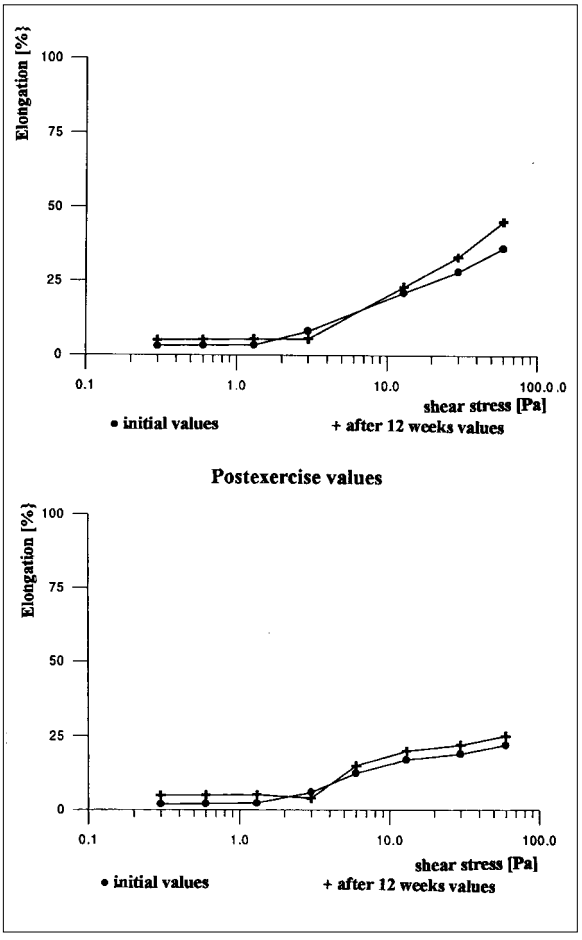

Figure 3. Comparison of the percentage elongation changes of the red cells: resting and postexercise, in the surgical group. Measured on entry and after 12 weeks of treatment.

a 400% increase in intracellular Ca^{2+}, resulting in the rigidity of the erythrocytes [7,14].

It has been hypothesized that hemoglobin and other intracellular proteins that are soluble in physiological conditions may become insoluble when ATP levels are diminished, followed by the rapid growth of intracellular Ca^{+} [6,7]. The result may be the formation of a gel layer separating the membrane from its cytoplasm, producing the deforma-

bility of erythrocytes and changing their mechanical properties. When the ATP level is normal, the shape and elasticity of the erythorcytes also return to normal during the incubation, which is mediated by adenosine.

It has been assumed that the deformability of erythrocytes may be attributed to the following factors:

1. their large surface and high surface/volume ratio, due to the biconcave disk shape. When erythrocytes take on a spherical shape, as for example in a hypotonic environment, their cellular surface area is reduced (the surface/volume ratio is lowered), and their ability to deform is thus limited.
2. the elasticity of the erythrocyte membrane. The extent of erythrocyte elasticity has not yet been measured, but according to some authors it may range from 10% to 15% [8,10,14].
3. the intracellular viscosity of the hemoglobin solution. In dehydration, as for instance in hypertonic cells, the elasticity of the red cells is reduced as a result of the increased hemoglobin concentration and intracellular viscosity. It has been suggested that the alteration of one or several of these factors produces the deformability of erythrocytes [8,10,14].

Red cell rigidity was also observed in our patients with atheromatosis and chronic ischemia of the leg. The deformability (elongation) of the red cells in these patients was significantly lower at the beginning of the study, as compared to the controls. The lowest parameters were observed in the group qualified for surgical revascularization. This observation may indicate a relationship between the degree of rigidity and the stage of atherosclerosis and ischemia of the leg.

RBC elasticity decreased further after walking exercise. This may be attributable to hypoxia, which increased as a result of flow decompensation in the patients with intermittent claudication. The relationship between hypoxia and RBC rigidity has been confirmed in numerous publications [8,11,15]. La Celle has postulated that the alterations taking place under these circumstances result from ATP binding by hemoglobin and the formation of a gel that causes the deformability and rigidity of erythrocytes [8,9,10].

The results of our studies support the observations made by other authors, that conservative treatment in patients with less advanced atherosclerosis and ischemia of the leg does not exert a significant influence on the hemodynamic conditions in the macrocirculation. Neither an increase in systolic pressure in the peripheral arteries nor an increase in the A/BI were observed.

According to clinical observations, the alleviation of symptoms may be largely attributed to favorable rheological changes resulting from the conservative treatment administered [16,17]. Both experimental and clinical studies have shown that regular physical exercise, including walking exercises, can modify many rheological parameters and lower the hematocrit value (fluidification anemia), fibrinogen level, and absolute blood and plasma viscosity [16,18]. It has been observed that the concentration of potassium and sodium in the erythrocytes of persons doing regular exercise does not change during graded walking exercises. By contrast, it increases in persons who do not exercise regularly. It may be hypothesized that the increase of potassium and sodium concentration in persons not adapted to regular physical effort is caused by the lowered capacity of the ion pump, which in turn may point to disorders in the energy regeneration mechanism in the erythrocytes [6,7,14]. Siefring and Roland hypothesized that impaired ATP production in the red cells makes it impossible to pump out calcium ions also. These ions can trigger the decomposition of the red cells' stroma proteins, causing membrane rigidity and the loss of elasticity in the erythrocytes [14,19,20,24].

The patients who received kinesitherapy were also administered Pentoxyphilline, a hemorheological drug that improves RBC elasticity and lowers blood viscosity by decreasing the fibrinogen level and blood platelet aggregation [21–23]. In vivo and in vitro studies have demonstrated that Pentoxyphilline has a significant impact on the level of adenine nucleotides in red cells [22,23,25]. In patients with chronic atheromatous ischemia of the leg, a dose-related increase in the ATP level in the erythrocytes has been observed. In animal experiments, orally administered Pentoxyphilline significantly increased the levels of ATP, the total concentration of adenine nucleotides, and the ATP/ADP ratio in the red cells. Similar effects of Pentoxyphilline have also been observed in humans [21–23,26].

In patients with seriously advanced atheromatosis and critical ischemia of the leg, successful surgical revascularization brought about symptomatic improvement. The changes observed were manifested mostly by an increased flow in the macrocir-

culation, reflected by the elevated systolic blood pressure in the peripheral arteries, and, as expected, by the simultaneous increase of the perfusion gradient pressure at the macro/microcirculation border.

Revascularization alone appears to be ineffective in correcting unfavorable rheological alteration, such as high blood viscosity, a high level of fibrinogen, or – as demonstrated in our study – erythrocyte rigidity [22,23,26]. In view of the available experimental and clinical observations, the persistence of these unfavorable rheological changes, including erythrocyte rigidity, may continue to hinder flow in the microcirculation in this group of patients, despite successful revascularization.

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