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Red blood cell deformability and aggregation in chronic venous disease patients with varicose veins

Odkształcalność i agregacja krwinek czerwonych u pacjentów z przewlekłą niewydolnością żylną kończyn dolnych z towarzyszącymi żylakami

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Introduction: Red blood cells' (RBC) rheological properties are disturbed in chronic venous disease (CVD). The aim of the study was to compare deformability and aggregation of erythrocytes taken from the varicose vein and the antecubital vein of patients with chronic venous disease.

Materials and Methods: Blood samples were taken from twelve CVD patients presenting clinical, aetiological, anatomical and pathological elements (CEAP) stages II and III. Blood was sampled from varicose veins and antecubital veins of patients (as control). Deformability and aggregation of RBC were analysed with a Laser-assisted Optical Rotational Cell Analyser (LORCA).

Results: A significant increase in deformability was found in varicose vein RBC for shear stress values 4.24, 8.23 and 15.96 Pa as compared to RBC from the antecubital vein. The aggregation index was significantly lower and aggregation halftime was significantly increased for RBC taken from antecubital veins than for RBC from varicose veins.

Discussion: In conclusion, RBC taken from varicose and antecubital veins of CVD patients are not entirely rheologically comparable and show different deformability and aggregation. Varicose vein RBC are more deformable and show a higher tendency for aggregation than antecubital vein RBC. Perhaps the deformability of varicose vein RBC has been increased as a compensation mechanism in subjects with CVD, due to increased resistance in their microcirculation.

Keywords: aggregation • deformability • chronic venous disease • red blood cells

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Abbreviations: **AI** – aggregation index; **AMP** – amplitude of aggregation; **au** – arbitrary unit (au); **CEAP** – clinical, aetiological, anatomical and pathological elements; **CVD** – chronic venous disease; **EI** – elongation index; **LORCA** – laser-assisted optical rotational cell analyser; **MDA** – malondialdehyde; **PVP** – polyvinylpyrrolidone; **RBC** – red blood cells; **T1/2** – aggregation halftime; **THR** – threshold shear rate.

Introduction

Over the last years extensive research has been performed on different aspects of red blood cell (RBC) aggregation and deformability. RBC deformability refers to the ability of the entire erythrocyte to deform and elongate in response to applied forces such as shear stress and secures the appropriate tissue perfusion. The aggregating property of RBC is a reversible phenomenon which leads to RBC rouleaux formation. RBC aggregation significantly influences blood viscosity *in vivo* [1,19].

RBC rheological properties can be affected by various pathophysiological processes and are disturbed in numerous pathological conditions including arterial hypertension, myocardial infarction and chronic venous disease (CVD) [6,15,16,21]. One of the manifestations of CVD is varicosis of lower extremities. Varicosis is a complex pathology associated with blood stagnation, venous hypertension, turbulence, and back-flow leading to pathological tortuosities of the vein wall [4, 14]. Such pathological changes can affect RBC mechanical properties.

RBC rheological changes in CVD patients were reported previously. However, a very limited number of studies have been performed to compare *in vitro* rheological behaviour of RBC taken directly from varicose veins and paired antecubital veins of CVD patients. Thus, the aim of the present study was to compare deformability and aggregation of red blood cells taken from the varicose vein and the antecubital vein of patients with CVD.

Materials and Methods

Fresh blood samples in sodium EDTA were taken from twelve patients (mean age 44.64 ± 14.75 years) suffering from CVD and presenting clinical, aetiological, anatomical and pathological elements (CEAP) stages II and III. The patients considered for the study were those attending the 2nd Chair of General Surgery, Jagiellonian University Medical College for the management of venous disease. The diagnosis of primary varicose vein was based on both the clinical examination and duplex scanning examination. The patients had a positive family history of CVD and the symptoms of disease were observed for more than one year. Additionally, in patients enrolled in the study fibrinogen level remained within the normal range and none of them had taken any medication at least two weeks before the study.

Blood was sampled from varicose veins and paired antecubital veins (as control) of CVD patients. Subjects gave their informed consent to participate in the study, which was approved by the Commission of Bioethics of the Jagiellonian University.

Deformability and aggregation of RBC were analysed with a Laser-assisted Optical Rotational Cell Analyser (LORCA, Mechatronics, The Netherlands) [9,10]. Every blood test was performed within thirty minutes after blood withdrawal. The measurements were carried out at 37°C according to the standard protocol.

RBC deformability was measured using 25 µl of blood diluted 1:200 with 0.14 mM/l polyvinylpyrrolidone medium (PVP, pH 7.4, osmotic pressure 300 mOsm/kg, viscosity 30 mPa, Sigma). Blood samples were poured into the gap between two coaxial glass cylinders. The rotating outer cylinder exerts a force (shear stress) on the suspension of cells. Increased shear stress (between 0.30 Pa and 59.97 Pa) causes the cell to elongate, which changes the dispersion of light (a diode laser: 670 nm, 4 mW) recorded by the sensor of the apparatus [10]. RBC elongation was measured with the elongation index (EI) calculated as EI=(A-B)/(A+B) where A and B are the vertical and horizontal axes of the ellipse, respectively.

Aggregation measurement was performed on a sample of 2 ml of whole blood. The instrument analyses the intensity of light scattered by the blood sample. The aggregation measurement is based on the detection of laser back-scattering from the sheared (disaggregated) to unsheared (aggregated) blood. RBC aggregation parameters were determined with a syllectogram, which is a curve illustrating the change in the light intensity of scattered light during 120 s corresponding to the process of aggregation [9]. The following aggregation measurements were performed: aggregation index (AI), aggregation halftime (T1/2) threshold shear rate (THR) and the amplitude of aggregation (AMP).

Statistical significance was determined by using a paired Student *t*-test. *p* values less than 0.05 indicated statistical significance.

Results

RBC deformability data are summarized in Table 1. An increased EI at a given shear stress indicates greater cell deformation and hence greater RBC deformability. A significant (p<0.05) increase in deformability was found in RBC taken from the varicose vein of CVD patients for shear stress values of 4.24, 8.23 and 15.96 Pa as compared to RBC from the antecubital vein.

Experimental results showed that the mean value of AI for RBC taken from antecubital veins of patients with CVD was 56.8 ± 3.7 and was lower than for RBC from varicose veins (61.3 ± 3.6) (Fig. 1A). The difference reached a significant level in statistical terms (p<0.05). As depicted in Fig. 1B, THR values were higher in RBC taken from varicose veins (177.4 ± 11.8 1/s) when compared to antecubital veins (170.4 ± 13.3 1/s). The differences were however insignificant. Compared to antecubital veins (3.28 ± 0.4 s) varicose vein RBC had significantly (p<0.05) lower T1/2 values (2.77 ± 0.4 s) (Fig. 1C). Additionally, AMP was higher in RBC samples taken from the antecubital vein (21.89 ± 1.31 au) in relation to the varicose vein RBC (21.09 ± 1.26 au) but the difference was not significant (Fig. 1D).

Table 1. Red blood cells (RBC) elongation index (EI) at different shear stresses for blood samples from varicose and antecubital veins

Shear stress [Pa]	EI	
	Antecubital vein	Varicose vein
0.30	0.024 ± 0.009	0.020 ± 0.011
0.58	0.030 ± 0.011	0.027 ± 0.012
1.13	0.085 ± 0.012	0.083 ± 0.009
2.19	0.208 ± 0.012	0.220 ± 0.009
4.24	0.328 ± 0.010	0.352 ± 0.009*
8.23	0.427 ± 0.009	0.451 ± 0.010*
15.96	0.525 ± 0.009	0.546 ± 0.010*
31.04	0.572 ±0.010	0.571 ± 0.012
59.97	0.599 ± 0.011	0.601 ± 0.011

Data are presented as X ± SD.
*** Statistically significant differences at significance level 0.05 between varicose and antecubital veins.**

Discussion

The pathophysiological and clinical aspects of RBC haemorheological alterations point out a special feature of blood rheology: haemorheological impairments might result from the disturbance of local homeostasis. During CVD blood stasis in lower veins leads to hypoxia and excess delivery of oxygenated free radicals [12, 14, 18]. Such changes may affect RBC aggregation and deformability [20, 22].

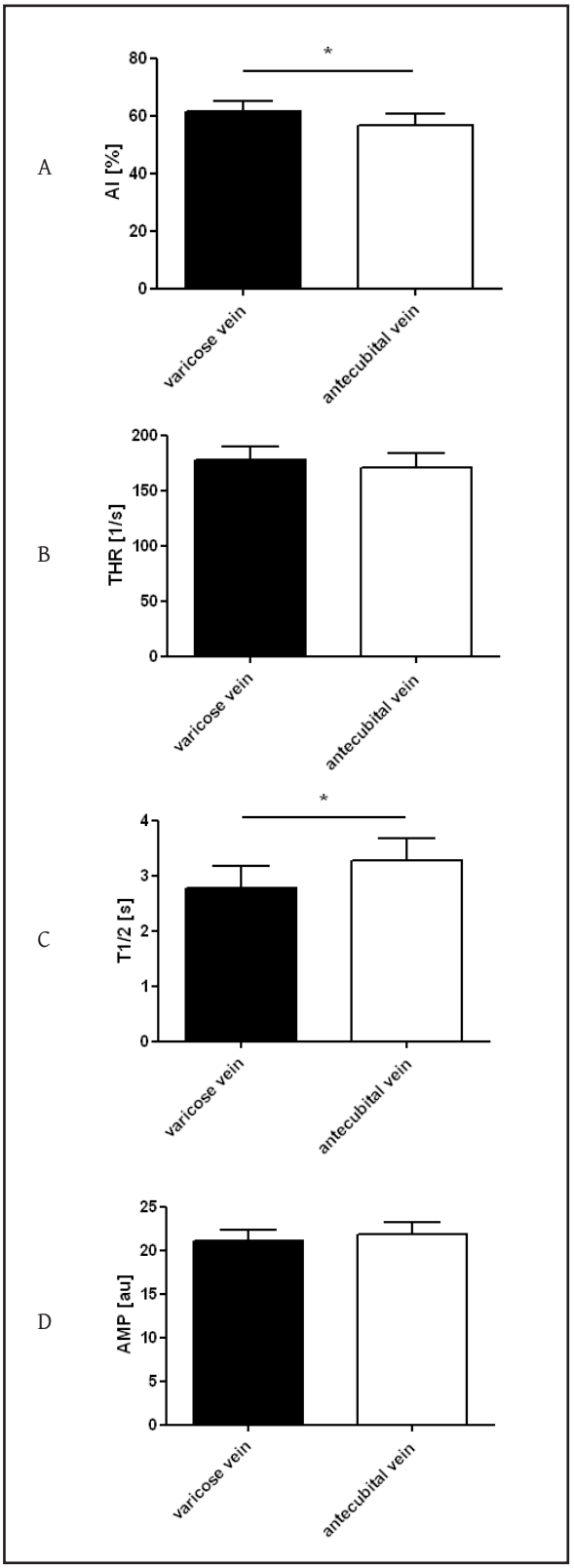

Fig. 1. Red blood cell (RBC) aggregation indices in blood samples from varicose and antecubital veins. (A) aggregation index (AI); (B) threshold shear rate (THR); (C) aggregation halftime (T1/2); (D) the amplitude of aggregation (AMP). Data are presented as X ± SD; au - arbitrary unit.
*** Statistically significant differences at significance level 0.05 between varicose and antecubital veins**

Haemorheological impairments in CVD were reported previously by other researchers [3-5,14]. To investigate chronic venous disease further, the present study aimed to find out whether RBC taken directly from the varicose vein differ in their deformability and aggregation from RBC taken from the antecubital vein of patients with CVD.

According to the results obtained, RBC derived from varicose veins were more deformable than RBC from antecubital veins of patients. Perhaps the deformability of varicose vein RBC has been increased as a compensation mechanism in subjects with CVD, due to increased resistance in their microcirculation [5]. This phenomenon may have some fundamental significance in CVD as increased RBC deformability ensures RBC adaptation to hydrodynamic forces. The cells easily move to different regions of the circulatory system, ensuring adequate peripheral blood flow. Le Dévéhat et al. [5] previously examined rheological properties of blood in patients with superficial venous insufficiency in the veins of the lower limbs. Two blood samples were obtained from the veins of the lower limbs of each subject: the first one at rest and the second after ten minutes of experimentally induced venous stasis. They observed that before and after stasis, RBC of patients were significantly less deformable than those of controls and they were found to be more altered in the patient group following stasis. The existing discrepancy between our results and those of Le Dévéhat et al. may be attributed primarily to the presence or absence of experimentally induced blood stasis but also to different equipment and techniques applied. Le Dévéhat et al. used a filtration method based on the initial flow rate conditions of blood sample suspension by using a Hanss haemorheometer with a Nuclepore membrane. Furthermore, the results obtained by Le Dévéhat et al. may not be directly applicable to *in vivo* situations and should only be considered to be relevant for *ex vivo* analysis of haemorheological parameters.

In the present work the phenomenon that varicose vein RBC were more deformable than cells from antecubital veins of patients was observed only at higher values of shear stress (4.24-15.96 Pa). At the other shear stress values EI did not differ significantly between the tested RBC. Hardeman and Ince [11] reported previously that depending on clinical conditions, various shear stress levels are observed in the different local regions of interest. Thus, various aspects of RBC deformation defects may manifest on different parts of the deformation curve.

With respect to RBC aggregation, the present study showed that aggregate formation was higher in RBC derived from varicose than from antecubital veins of patients determined with the aggregation index. On the other hand, the strength of aggregates was not affected (threshold shear rate values analysis). At the same time, when comparing varicose with antecubital vein RBC, there was a statistically significant drop in the value of T1/2, indicating a faster rate of the RBC aggregation process in varicose veins. These results demonstrated the early formation of RBC aggregates in some low shear conditions such as in varicose veins. This is consistent with the previous experiments by Le Dévéhat et al. [5], who found that disturbances in aggregation indices in patients with superficial venous insufficiency in the veins of the lower limbs were more pronounced after stasis.

The different aggregation profile of antecubital and varicose vein RBC of CVD patients may be directly linked to local factors observed in varicose veins. The main factors that influence RBC aggregation include *inter alia* the levels of plasma macromolecules including fibrinogen and RBC properties such as shape and membrane charge [2, 17]. In our patients rated II and III according to the CEAP classification the level of fibrinogen remained within the normal range. This suggests the involvement of other factors. It was reported previously that vasodilation and venous stasis cause relative hyperviscosity and hypoxia. Due to hypoxia and pH decrease leucocytes are activated. Consequently the cells release toxic metabolites and oxygen free radicals [8]. These molecules may easily affect RBC mechanical properties. Flore et al. [7] showed that in patients with chronic venous insufficiency the levels of oxygen free radicals in blood samples taken from foot veins were higher in patients with varicose veins than in the control group. On the other hand, Jacob et al. proved that experimentally induced stasis did not influence malondialdehyde (MDA) levels and they did not find differences in MDA levels between blood samples taken both from arms and legs of patients with varicose veins [13]. MDA is one of the end products of lipid peroxidation.

It is not possible to explain the exact reason for enhanced RBC aggregation in varicose veins of CVD patients. Simultaneous measurements of the above-mentioned aggregation parameters and the level of oxygen free radicals in varicose vein blood and in varicose vein wall samples may contribute more readily in recognizing the issue.

In conclusion, RBC taken from varicose and antecubital veins of CVD patients are not entirely rheologically comparable and show different deformability and aggregation. Varicose vein RBC are more deformable and show a higher tendency for aggregation than antecubital vein RBC.

The authors have no potential conflicts of interest to declare.

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