Hemorheological investigations in carotid artery stenosis and in critically ill patients

Ph.D. dissertation

Kinga Totsimon, M.D.
Clinical Medicine Experimental Cardiology

Program leader:
Prof. Kalman Toth, M.D., Sc.D.

Project leaders:
Zsolt Marton, M.D., Ph.D.
Peter Kenyeres, M.D., Ph.D.

1st Department of Medicine, University of Pecs
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Table of contents

1. List of abbreviations

ACEi	angiotensin-converting enzyme inhibitor
AI	aggregation index
Apache	Acute Physiology and Chronic Health Evaluation
ARB	angiotensin-receptor blocker
AS	atherosclerosis
CAS	carotid artery stenosis
CI	confidence interval
CRP	C-reactive protein
e.g.	exempli gratia
EI	elongation index
EI_{max}	maximal elongation index
GOT	glutamic-oxaloacetic transaminase
Hct	hematocrit
hgb	hemoglobin
HR	Hazard ratio
i.e.	id est
ICU	intensive care unit
INR	international normalized ratio
LDL	low density lipoprotein
LORCA	Laser-assisted Optical Rotational Cell Analyzer
LOS	length of stay
MAP	mean arterial pressure
MOF	multiple organ failure
PCT	procalcitonin
pH	power of hydrogen
PV	plasma viscosity
RBC	red blood cell
SAPS	Simplified Acute Physiology Score
SBP	systolic blood pressure
SD	standard deviation
SS ½	shear stress required for half of EI_{max}
t ½	aggregation half time
TIA	transient ischemic attack
WBC	white blood cell
WBV	whole blood viscosity
γ	threshold shear rate

2. Prologue

2.1. Clinical role of hemorheology

Hemorheology investigates the blood flow, and the flow properties and interactions of blood cells. In the past few decades many clinical researches suggested that hemorheological alterations can be determinative in vascular diseases, in some haematological diseases, and also in critical conditions. Deterioration of these parameters could result in tissue hypoperfusion and disturbances in the microcirculation [1-3].

Atherosclerosis, as the basis of the development of vascular diseases, also has an important rheological aspect. The results of Edinburgh Artery Study showed the association of fibrinogen, whole blood and plasma viscosity with carotid intima-media thickness. In addition, hemorheological alterations occur prior to the development of vascular lesions indicating that they can promote the atherosclerotic process [1-4].

The coronary vessel system is a unique part of the circulation due to the periodic change in blood flow and the extraordinary narrow capillaries. Under critical circumstances rheological variables can have an important role in myocardial perfusion. Numerous prospective epidemiological studies (Framingham Study [5], the Edinburgh Artery Study [4], the Monica Project [6-7], the Honolulu Heart Program [8], the Physicians' Health Study [9], the Caerphilly Study and the Speedwell Study [10-11]) have described that besides conventional causes, such as hypertension, diabetes mellitus, smoking and hypertriglyceridaemia; hematocrit, fibrinogen and viscosity are independent cardiovascular risk factors. Furthermore, the

deterioration of rheological factors could predict acute events and they are associated with unfavourable outcome [1-3, 12, 13].

Impaired rheological properties of the blood are also cerebrovascular risk factors. By their influence on thrombogenesis and atherosclerosis, they can contribute to the decrease of cerebral blood flow in acute ischemic stroke. Owing to the increased concentration of acute phase proteins, such as fibrinogen, they can also play a role in poor outcome. Persisting hemorhelogical alterations after a stroke can be prone to recurrent event; therefore their observation could be important in secondary prevention [1-3].

In critical conditions, when hemodynamic instability develops, hemorheological parameters could be essential in appropriate tissue perfusion. In sepsis, that is associated with profound microcirculatory abnormalities and cell damage, deteriorated microrheological properties contribute to the worsening of tissue oxygenation [1-3, 74-76]. Macrorheological factors can be impaired also in nonseptic patients [90]. Previous findings showed that these alterations can refer to the prognosis of the patients [64-72].

2.2. Clinical role of the hemorheological parameters

Hematocrit (Hct)

Hematocrit, as the percentage of red blood cells in whole blood is frequently used in the clinical practice. Hematocrit has a double role: firstly, it reflects the oxygen carrying capacity of blood since higher hematocrit usually correlates with higher hemoglobin concentration and higher oxygen binding capacity. Secondly, the hematocrit value has a logarithmic relation with blood viscosity, thus it is a major determinant of flow resistance.

In vascular diseases elevated Htc has been described. Epidemiological findings have indicated Hct as a cardiovascular risk factor, it is predictive of acute myocardial infarction, unstable angina and stroke, and it is also associated with the extent of coronary and cerebral atherosclerosis. In critically ill patients not only elevated, but decreased Hct can also impair tissue oxygenation by lower hemoglobin concentration and oxygen binding capacity. Its importance is showed by the fact that Hct is a component of disease severity classification systems in Intensive Care Unit [1-3].

Fibrinogen

Higher plasma fibrinogen concentration increases plasma and whole blood viscosity, facilitates red blood cell and platelet aggregation and promotes blood clotting. It is correlated with the extent of coronary atherosclerosis and it is also elevated in chronic vascular disease. It is a risk factor of cardiovascular and cerebrovascular events associating with unfavourable outcome. In sepsis it contributes to microcirculatory disturbances and tissue hypoxia by the enhancement of RBC aggregation [1-3].

Whole blood and plasma viscosity

Blood viscosity, the intrinsic friction of the circulating blood, is a major determinant of flow resistance. Whole blood viscosity is influenced by the hematocrit, plasma viscosity, and at low shear rates strongly by red blood cell aggregation, while at high shear rates high deformability becomes determinative. Plasma viscosity is determined by plasma proteins such as fibrinogen, globulins, and the triglyceride level. Blood viscosity is an important

determinant of flow resistance. Increased blood viscosity results in worsened circulatory insufficiency, although under normal conditions, vasomotor control mechanisms try to compensate hemorheological alterations and maintain adequate blood supply.

Increased blood viscosity could promote atherosclerosis, it is a cerebrovascular risk factor by reducing cerebral blood flow; furthermore, epidemiological studies have found that it is also a risk factor for cardiovascular events [1-3, 15].

Red blood cell aggregation

Red blood cell aggregation develops reversibly at stasis or at low shear conditions. It is influenced by hematocrit, membrane surface adhesion molecules, concentration of plasma macromolecules (e.g. large plasma proteins, lipids; neutral polymers in vitro), red blood cell aggregability (the intrinsic cell characteristics), and red blood cell deformability.

Elevated red blood cell aggregation has been shown in chronic clinical conditions such as diabetes mellitus, cardiovascular diseases, multiple myeloma, malignant and autoimmune disorders. In sepsis the higher level of acute phase protein fibrinogen can result in enhanced RBC aggregation [1-3, 16]. In spite of these observations, the connection between aggregation and pathological disorders, and the mechanisms behind them are not fully understood.

Red blood cell deformability

The resting red blood cell has a biconcave discoid shape with a diameter of approximately 7-8 µm. The diameter of the capillaries average 3-5 µm, it is essential for the cell to undergo rapid, large-scale deformations to enter and pass the microcirculation and nutritive capillaries. Red blood cells have an excess surface area of about 40% compared to a sphere of equivalent volume that allows extensive deformation to shapes far from spheres. It depends on the membrane viscoelastic properties, the internal viscosity of red blood cells, the surface-volume ratio, and cell morphology [14, 17-21].

RBC deformability can be deteriorated by the alterations of membrane structure proteins and cytoskeleton. Causes can be genetic (hemoglobinopathies, enzyme deficiencies of RBC metabolism, etc.), mechanical trauma (artificial heart valve), oxidative damages (e.g. ischemia/reperfusion injury, activated leukocytes), or disturbances of blood properties (e.g. osmotic pressure, pH). Impaired deformability can also be associated with vascular diseases and sepsis [1-3].

2.3. Methodology

Hematocrit (Hct)

Hct was measured in a Haemofuge microhematocrit centrifuge (Heraeus; Germany) using native capillaries. Measurements were performed at room temperature (22±1°C) [22-24].

Plasma fibrinogen

Venous blood sample was collected into a Na-citrate contained Vacutainer tube. Plasma fibrinogen concentration was determined by Clauss method [5].

Plasma and whole blood viscosity

Plasma viscosity (PV) was measured with a Hevimet 40 capillary viscometer at 37 °C (Hemorex Ltd., Budapest, Hungary [13]). Plasma was collected after whole blood sample centrifugation for 10 minutes at 1500g.

Figure 1: Hevimet 40 viscometer

In capillary type viscometers viscosity is measured based on the principle of measuring flow rate through a glass tube of specified dimensions. Hevimet 40 consists of 40 diodes besides a vertical glass tube, where the height of each diode is known. The height of the fluid column is registered according to time. When fluid sample is injected into the system and released to flow out, the flow maintaining hydrostatic pressure and shear stress at each point can be calculated. Based on the ratio of height and time, flow velocity and shear rate is determined. Viscosity is calculated from the shear stress and shear rate data points. Plasma, in contrast to whole blood, is Newtonian fluid thus its viscosity is an intrinsic property of the liquid itself. Therefore, plasma viscosity is independent of shear rate and there is no need to determine it at different defined shear rates [13, 22-25].

Whole blood viscosity (WBV) was also determined with Brookfield DV-III Ultra LV cone-plate rotational viscometer (Brookfield Engineering Laboratories Inc, Middleboro, USA [12]). Measurements were performed at 37 °C.

Figure 2: Brookfield DV-III viscometer

Rotational viscometers are composed of two surfaces: a static and a rotating one. The sample is placed in a narrow gap between the surfaces. During the function, one of the

elements rotates at various speeds thereby producing specific shear rates. The cone-plate configuration measures shear stress with the torque measuring system. It is also important that unlike plasma, whole blood is a non-Newtonian fluid that means its viscosity is dependent on the shear rate at which it is measured. We measured whole blood viscosity at 15 different shear rates from 400 to 50 s^{-1} [22-24, 25, 26].

Red blood cell aggregation

Red blood cell aggregation measurements were carried out with a LORCA aggregometer (Laser-assisted Optical Rotational Cell Analyzer; R&R Mechatronics, Hoorn, The Netherlands) using laser light back-scattering syllectometry. During the measurements oxygenated blood samples were used, and measurements were performed at 37°C.

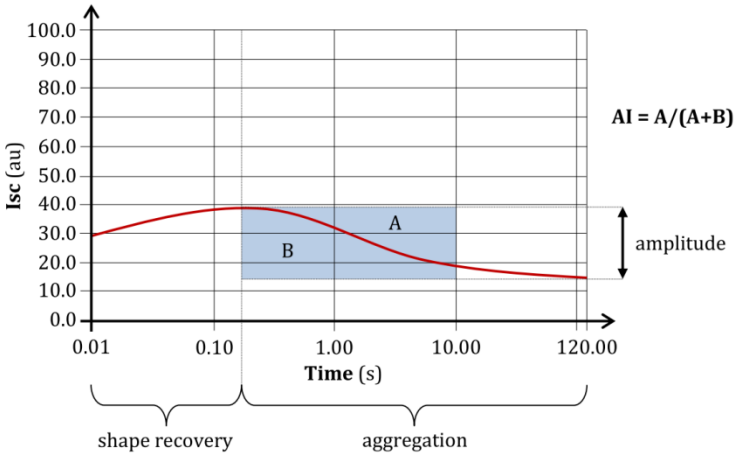


Figure 3: Syllectogramm
Source: Andras Toth., PhD dissertation.
University of Pecs, Medical School, 2014.
With the Author's approval.

LORCA aggregometer uses the process called "syllectometry", which measures the decrease in light backscatter. Red blood cells are placed in the gap of the instrument and disaggregated at a high shear rate (500 s^{-1}), which reduces rapidly to zero. At this moment sheared and elongated red blood cells lose their ellipsoid morphology and recover their

normal biconcave shape, the backscattering of laser light suddenly increases. Due to rouleaux formation and 3D-aggregates, it is immediately followed by a decrease of intensity and then it - referring to larger aggregates - reflects less light than single cells. For the characterization of the process aggregation index (AI; from the areas A and B of the diagram (AI=A/A+B) during the first 10 seconds of the measurement) and t½ (time that is needed to peak intensity of aggregation is reduced by half) were calculated. RBC disaggregation threshold shear rate (γ) was determined, which is the smallest shear rate required for the complete disaggregation of erythrocytes [22-24, 27-30]. Increased red blood cell aggregation is characterised by higher AI, γ and lower t½.

Red blood cell deformability

In our study we measured red blood cell deformability with LORCA ektacytometer.

Figure 4: LORCA

In LORCA ektacytometer there are two cylinders: the inner cylinder (bob) is static, while the outer cylinder (cup) rotates driven by a motor. Blood sample was suspended in a high viscosity (28-32 mPas) polyvinylpyrrolidone solution and injected into the gap between the two cylinders. A laser beam transversing the suspension creates a diffraction pattern on a diaphragm that is recorded and analysed by video camera and a computer controlled ellipse

fitting. At rest red blood cells show circular diffraction pattern, and parallel to the applied shear stress it becomes elliptical as cells deform and elongate.

For analysis, elongation index (EI) was calculated as the (length - width) / (length + width) of the pattern for each shear stress (SS) at 9 different shear stresses (from 0.3 to 30 Pa). For data analysis, Lineweaver-Burke nonlinear curve fitting technique was used to calculate the maximal EI (EI_{max}) value at extrapolated infinite shear and the shear stress value required for half of EI_{max} ($SS_{1/2}$). EI ranges from 0 to 1, 0 refers to an undeformed, randomly oriented RBC, and EI increases with cell deformation. Higher EImax refers to higher deformation ability, while RBCs with higher SS1/2 are harder to deform. Measurements were performed at 37°C [22-24, 31-36].

3. Focus and aims of the studies

1. Hemorheological alterations in carotid artery stenosis

Carotid artery stenosis (CAS) is not only an important risk factor of cerebrovascular events but it can also indicate generalized atherosclerosis. Previous investigations showed that hemorheological parameters are altered in CAS and in chronic cerebrovascular disorders as well, but it is controversial whether the impairment of blood rheology is the late consequence of cerebrovascular events or the marker of carotid atherosclerosis. This study had the aim of determining the relationship among hemorheological parameters, stenosis and atherosclerosis both in patients who had cerebrovascular event before and who have not had it.

2. The relationship between hemorheological parameters and mortality in critically ill patients

The prognostic scoring systems for mortality of intensive care patients estimate clinical outcome using several physiological and biochemical parameters. Although predictive value of these systems is considered adequate, their accuracy is controversial. In altered hemodynamic conditions of critically ill patients, hemorheological variables may play a significant role in appropriate tissue perfusion. However, except for hematocrit in Apache, predicting models do not contain any hemorheological parameters, moreover the possibility of being prognostic markers is still unclear. We investigated if hemorheological parameters are altered in critical status and if they could be markers of mortality.

4. Hemorheological alterations in carotid artery stenosis

4.1. Introduction

Stroke and carotid artery stenosis

Stroke is one of the most life-threatening conditions that affects 17 million people worldwide a year. In spite of the development of stroke therapy, it is estimated that 23 million people will suffer from stroke with almost 7.8 million deaths globally by 2030. As the medical treatment improves and the number of stroke survivors increase, functional impairment in post-stroke conditions will become a more remarkable problem. Almost one third of stroke survivors are affected by chronic disability and half of the elderly stroke sufferers have cognitive deficits [37-39].

Stroke, which may be hemorrhagic or ischemic, is mainly caused by ischemia due to occlusion or stenosis of the blood vessel in circa 87% of stroke events and only the remaining cases are related to haemorrhage [41]. In the pathophysiology of ischemic stroke thrombosis plays an essential role. It can cause stroke by carotid atherosclerotic plaque rupture (in approximately 70–80% of cases) and by systemic embolism of a cardiac thrombus (in about 20-30% of cases, mostly in patients with atrial fibrillation) [37-39].

In stroke prevention two main strategies can be applied: first, the ‘mass’ approach that focuses on the population (decreasing risk factors with epidemiological methods, like reducing salt intake and smoking, removing trans fat) and second, the ‘high risk’ approach that identifies the individuals with risk factors and treats only them. In risk calculation several risk models exist that are based on traditional risk factors. Randomized controlled

trials and epidemiological studies, like the Framingham study, showed that hypertension, diabetes mellitus, hypercholesterolemia, cigarette smoking, atrial fibrillation, and carotid stenosis are independently associated with the incidence of stroke and treating them reduced the incidence of ischemic stroke/transient ischemic attack (TIA). However, only 60-80% of ischemic strokes can be explained by these factors, in addition the carotid substudy of Northern Manhattan studies suggested that conventional risk factors explain only a minority of carotid plaque alterations. Identification of nontraditional risk factors can help to understand the development of atherosclerotic plaque, adding them to the risk models can result in a more accurate risk clarification and treating them can reduce the incidence of stroke events. Several investigations, such as the Interstroke study, identified nontraditional risk factors, like obesity and metabolic syndrome, psychosocial stress and depression, ratio of apolipoprotein B to A1, sleep apnea, chronic inflammation, chronic kidney disease (CKD), nutrition/diet, environmental factors, and alcohol abuse [40-47].

Carotid artery stenosis, (CAS) which is defined by a stenosis of ≥50% in the region of the bifurcation of the extracranial internal carotid artery, affects approximately 10% of the elderly population (age ≥ 80 years) [48]. It causes more than 10% of all strokes, in addition the annual risk of ipsilateral stroke is about 2% in patients with asymptomatic CAS. Furthermore asymptomatic CAS can be a better indicator of generalized atherosclerosis than stroke risk due to the average annual risk of non-stroke death (mostly ischemic heart disease) being higher than of stroke risk. Carotid bruit itself has a significant impact, the rate of cardiac death or myocardial infarction in patients with carotid bruit is approximately twice compared with those without bruits. Interestingly, the extent of the carotid disease refers more to coronary death than its severity [49-51].

Hemorheology in cerebrovascular diseases

Based on previous findings, hemorheological parameters can indicate the extent of coronary and cerebral atherosclerosis [52]. Hemorheological parameters can also correlate with the degree of CAS, both in symptomatic and asymptomatic patients [53-55].

In chronic cerebrovascular disorders chronic hyperviscosity and increased fibrinogen level are present; furthermore, impaired deformability and elevated RBC aggregation were also found [1, 52, 56, 57]. Previous studies in the past 30 years suggest that altered hemorheology can correlate with the degree of carotid artery stenosis; hematocrit, plasma fibrinogen concentration, plasma viscosity, whole blood viscosity, and RBC aggregation can also be involved [52, 58]. Other publications have investigated asymptomatic patients to reveal the possible link between rheology and stenosis, and to assess the role of rheology in early atherosclerosis, although these findings have remained controversial. While plasma viscosity was found as a possible marker of atherosclerosis and carotid thickening [59], and some findings have supposed that RBC aggregation and fibrinogen can promote plaque formation [60], others have suggested that hemorheological parameters only have a minor role in early atherosclerosis [61].

It is not clear whether alterations in blood rheology are the late consequences of cerebrovascular events or the markers of carotid atherosclerosis. This study investigates the relationship among hemorheological parameters, stenosis and atherosclerosis both in symptomatic and asymptomatic cerebrovascular patients.

4.2. Subjects and Methods

Patients and study design

107 patients (44 males, 63 females, mean age 64±6 years) were recruited in the study. Patients with history of coronary artery or peripheral artery disease were excluded. 42 patients had ischemic stroke (proved by Computed Tomography) or transient ischemic attack in their history (symptomatic group). Based on carotid ultrasonography, patients were divided into non-stenotic group (<50% in diameter stenosis) and stenotic group (>=50% in diameter stenosis). Non-stenotic patients were further divided into three groups: (1) negative group with no evidence of carotid artery stenosis or atherosclerosis (11 patients, mean age 62±3), (2) evolving atherosclerosis (AS) group (~1-10% in diameter stenosis, 21 patients, mean age: 64±5), and (3) minimal stenosis (10-49%, 24 patients, mean age: 65±7); and stenotic patients into two groups: (4) moderate CAS (50-69%, 36 patients, mean age: 66±6) and severe CAS or occlusion (70-100%, 15 patients, mean age: 63±5). Laboratory measurements were performed within 3 months (±6 months) before or after ultrasonography. Demographic characteristics, risk factors, and therapy are shown in Table 1. Based on smoking habits current smoker group and current non-smoker group were defined, after that current non-smokers were divided into ex-smokers and never-smokers.

The study was approved by the Regional Ethics Committee of the University of Pecs and all subjects signed an informed consent before recruitment.

	Asymptomatic	Symptomatic	Non-stenotic	Stenotic
N	65 (60%)	42 (40%)	55 (52%)	52 (48%)
Male/female	23/42	21/21	18/37	26/26
Age (years)	65.2±6	63.1±6	63.6±6	65.1±6
Hypertension	51 (78%)	35 (90%)	43 (78%)	46 (88%)
Diabetes mellitus	14 (21%)	14 (33%)	10 (18%)	18 (34%) *
Smoking	5 (7%)	11 (26%)	7 (12%)	9 (17%)
Stroke or TIA	0 (0%)	42 (100%) *	17(31%)	25 (48%)
Antiplatelets	37 (57%)	40 (95%) *	30 (54%)	47 (90%) *
ACEi/ARB	40 (62%)	37 (88%) *	34 (62%)	43 (83%) *
Diuretics	26 (40%)	16 (38%)	22 (40%)	20 (38%)
β-blockers	30 (46%)	23 (54%)	25 (45%)	28 (54%)
Ca-channel blockers	23 (35%)	19 (45%)	19 (34%)	23 (44%)
Statins	33 (56%)	31 (74%) *	27 (49%)	37 (71%) *

Table 1: Demographic characteristics, risk factors, and therapy of patients.
** Represents significant differences p<0.05.*

Laboratory measurements

Blood samples were taken from the antecubital vein. Routine laboratory (blood sugar level, uric acid, cholesterol, triglyceride, total protein, albumin, C-reactive protein, and complete blood count) and hemorheological parameters were determined. Blood samples for hemorheologic measurements were collected into Li-heparin-coated Vacutainer tubes. Hemorheological measurements were carried out within 2 hours after blood sampling [2, 22-24], according to the above mentioned methodological description (see page 10-13).

Statistical analysis

Statistical analysis was performed with IBM SPSS statistical software version 22. Data are expressed as means ± SD. Differences between categorical variables were investigated with chi-squared test. Difference among groups for variables that were considered as normal distribution with Shapiro-Wilk test was evaluated by one-way ANOVA and Dunnett post hoc test. Nonparametric Mann-Whitney U-test was used for non-normally distribution variables. Significance level was defined as $p < 0.05$.

4.3. Results

In routine laboratory examinations, cholesterol and LDL were significantly higher in asymptomatic patients than in symptomatic patients and also in the non-stenotic group compared to the stenotic group. Albumin was reduced in the asymptomatic group and hgb was increased in smokers, but there were no differences in fibrinogen level or other routine laboratory tests (Table 2).

n	cholesterol (mmol/l)	LDL (mmol/l)	albumin (g/l)	fibrinogen (g/l)	hgb (g/l)
Asymptomatic (55)	5.49 ±1.3	3.47±1.2	45.3±2.4	3.16±0.6	142.7±14.9
Symptomatic (52)	4.97±1.3 *	2.68±1.0 **	46.4±3.3 *	2.99±0.7	143.6±15.9
Non-stenotic (65)	5.56±1.3	3.43±1.2	45.39±2.8	3.69±0.6	141.3±15.0
Stenotic (42)	5.03±1.3 *	2.88±1.1 *	46.09±2.8	3.09±0.6	144.8±15.0
Non-smokers (83)	5.14±1.3	3.06±1.2	45.68±2.8	3.06±0.6	141.1±14.7
Smokers (16)	5.65±1.3	3.24±0.9	46.88±2.9	3.18±0.6	153.4±15.4 **

*Table 2: Differences between certain routine laboratory measurements (cholesterol, low density lipoprotein, albumin, fibrinogen, and hemoglobin). Values are means ± SD. *p<0.05, **p<0.01*

Hematocrit was not different between non-stenotic and stenotic group, neither between asymptomatic and symptomatic group, while in asymptomatic patients non-stenotic group had significantly lower Hct level than stenotic group (Figure 5).

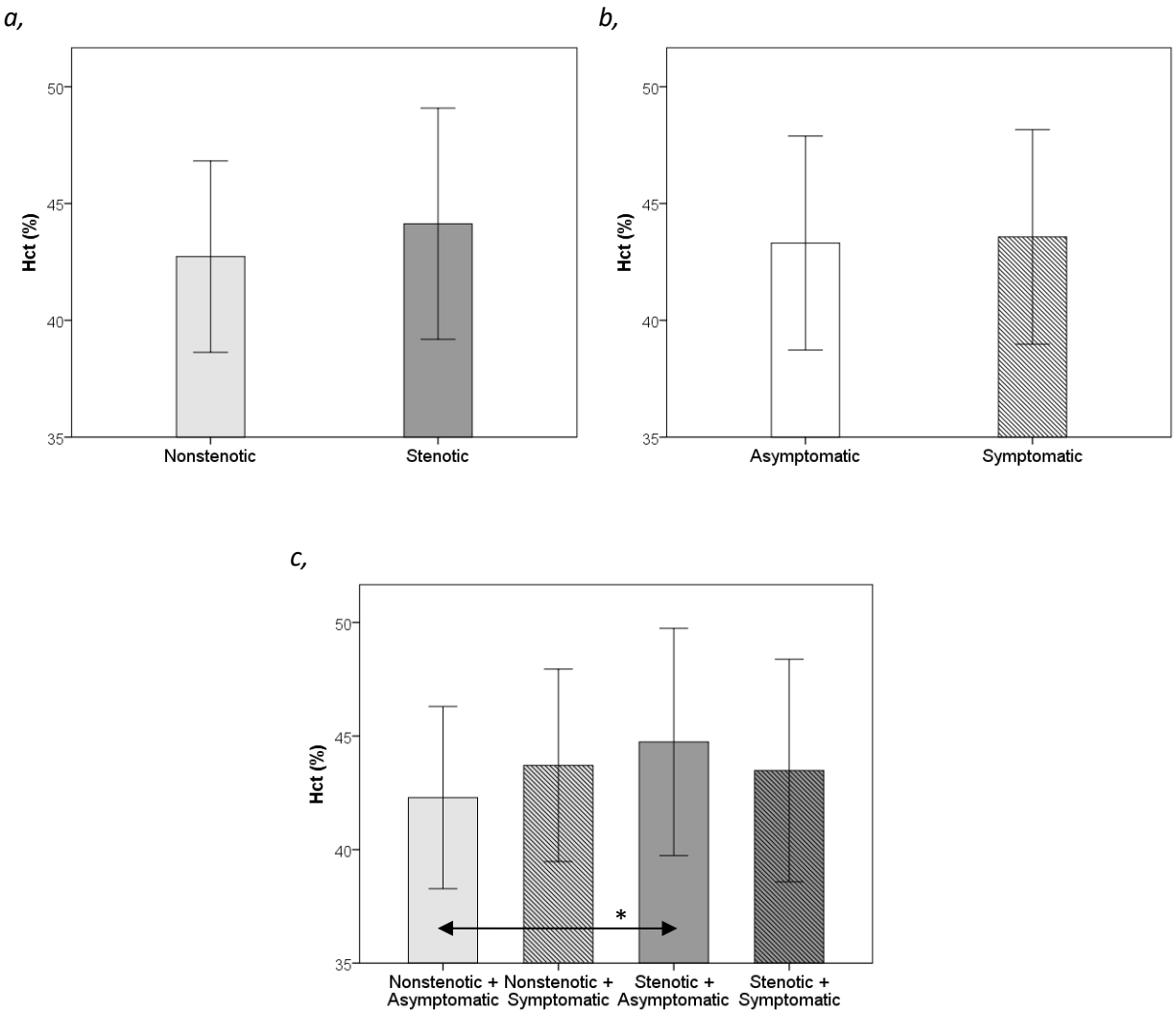


*Figure 5: Differences between hematocrit levels (a, nonstenotic – stenotic; b, asymptomatic – symptomatic; c, subgroups compared to nonstenotic and asymptomatic patients). Values are means ± SD. *p<0.05*

Whole blood viscosity (at every shear rates) was higher in stenotic group than in non-stenotic group, and also in symptomatic group compared to asymptomatic group. During subgroup analysis we found that non-stenotic patients without cerebrovascular event had significantly lower viscosity than both asymptomatic patients with stenosis and symptomatic patients with (at all shear rates) or without (only at certain shear rates) stenosis (Figure 6).

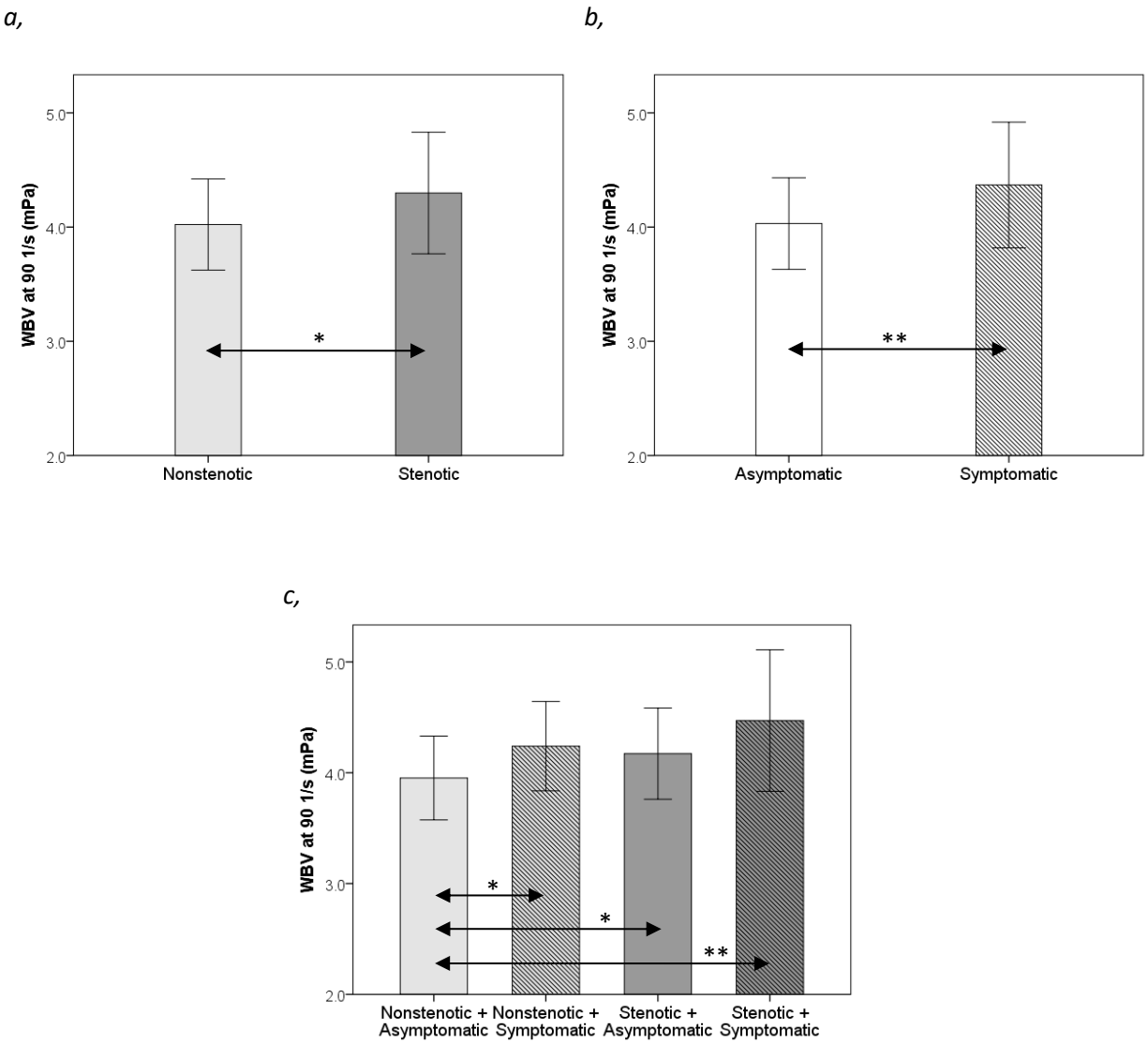


*Figure 6: Differences between whole blood viscosity levels (a, nonstenotic – stenotic; b, asymptomatic – symptomatic; c, subgroups compared to nonstenotic and asymptomatic patients). Values are means ± SD. *p<0.05, **p<0.01*

Plasma viscosity was significantly lower in asymptomatic patients compared to symptomatic patients. In the asymptomatic non-stenotic group PV was significantly lower than in the symptomatic non-stenotic group, and also lower than in the symptomatic stenotic group (Figure 7).

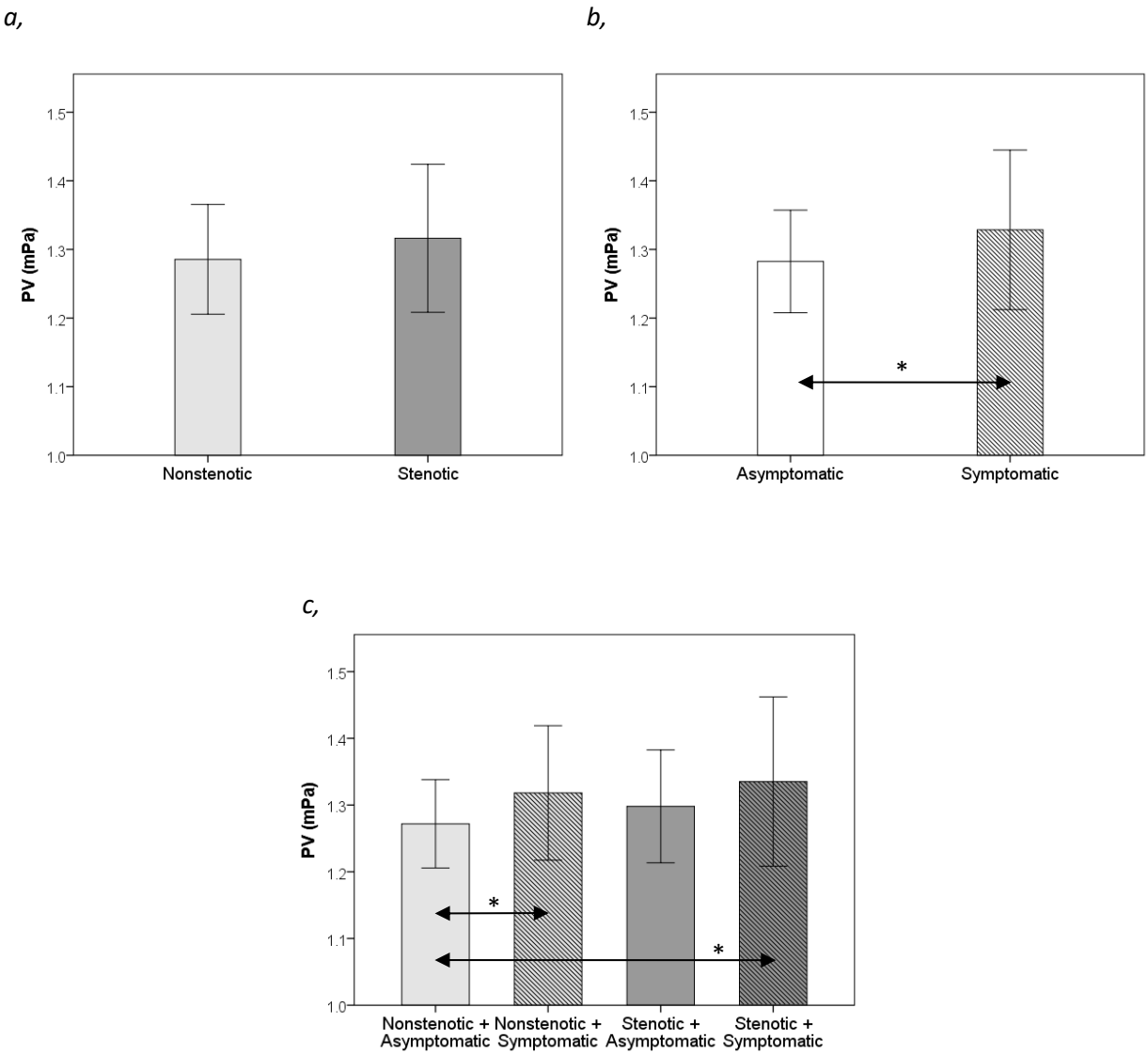


*Figure 7: Differences between plasma viscosity levels (a, nonstenotic – stenotic; b, asymptomatic – symptomatic; c, subgroups compared to nonstenotic and asymptomatic patients). Values are means ± SD. *p<0.05*

Evaluation of the results of red blood cell aggregation is controversial. There were differences neither in Aggregation Indexes (AI), nor in threshold shear rates (γ) regarding stenosis and symptoms. However, the value of t½ was lower in the stenotic group than in the non-stenotic group (nonstenotic patients: 1.77±0.5, stenotic patients: 1.63±0.6, $p<0.05$), what refers to a higher red blood cell aggregation.

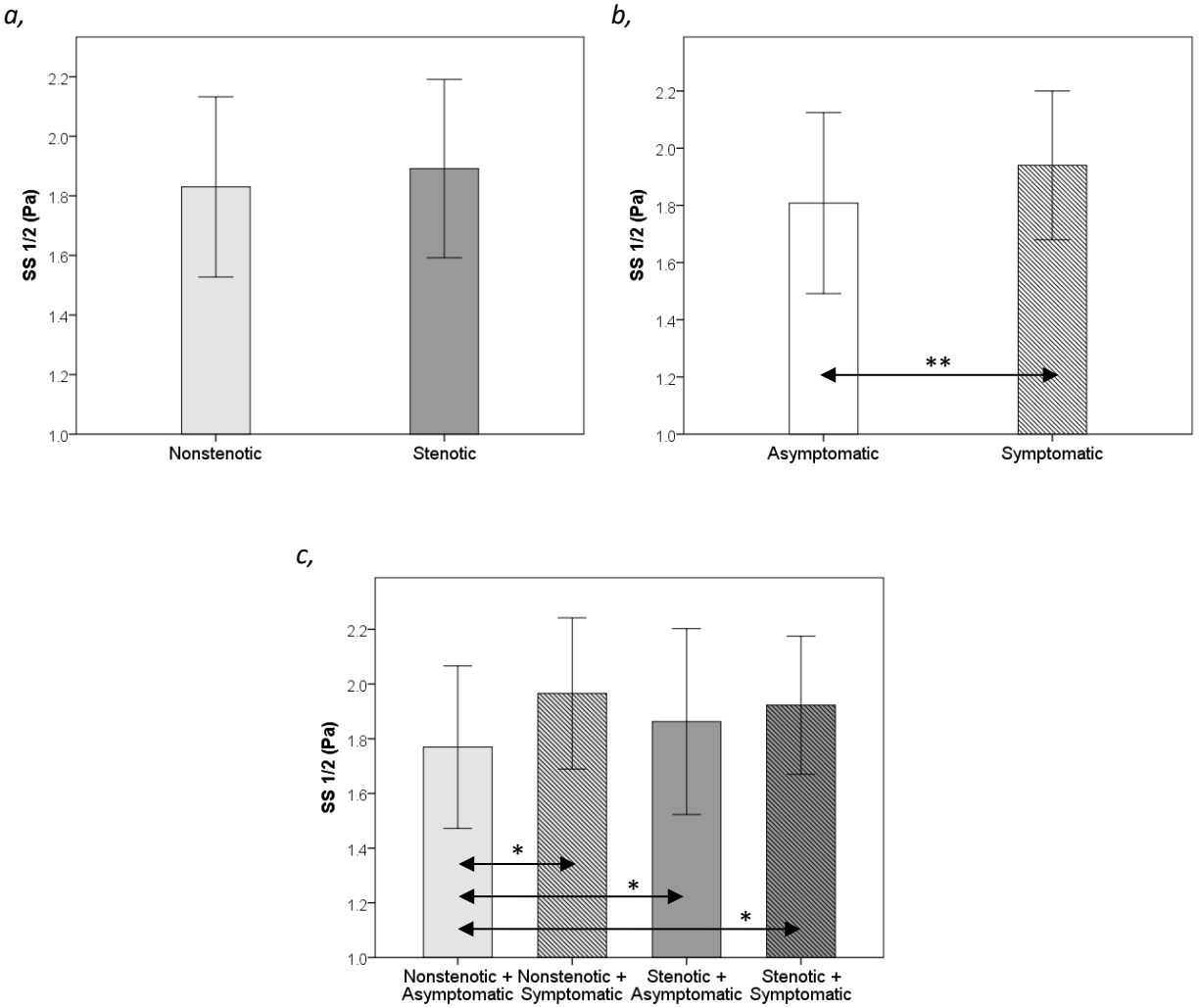


*Figure 8: Differences between red blood cell deformability (SS ½) levels (a, nonstenotic – stenotic; b, asymptomatic – symptomatic; c, subgroups compared to nonstenotic and asymptomatic patients). Values are means ± SD. *p<0.05, **p<0.01*

Worse red blood cell deformability (EI from 0.3 to 16.87 shear stresses and $SS_{1/2}$) was found in the symptomatic group compared to the asymptomatic group. Subgroup analysis showed RBC deformability of asymptomatic non-stenotic patients better than deformability of the three other groups (Figure 8).

EI

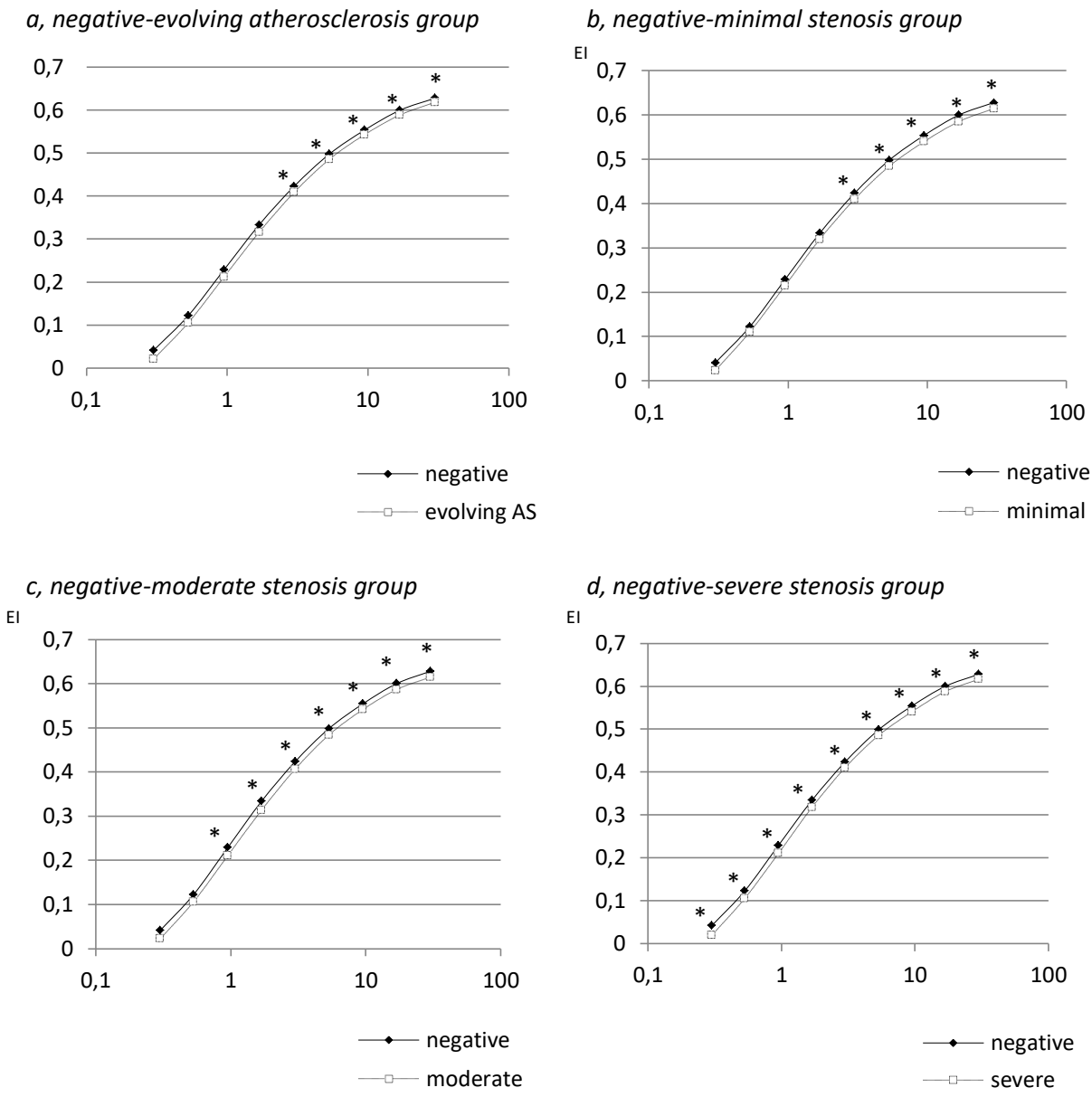


*Figure 9: Differences in red blood cell deformability among CAS groups. Values are means. *p<0.05*

Figure 9 and 10 represent data of the CAS subgroups. Impaired red blood cell deformability and elevated PV were found in the evolving AS and the CAS groups compared to the negative group. There was no difference between non-negative groups, neither between the moderate and the severe CAS group.

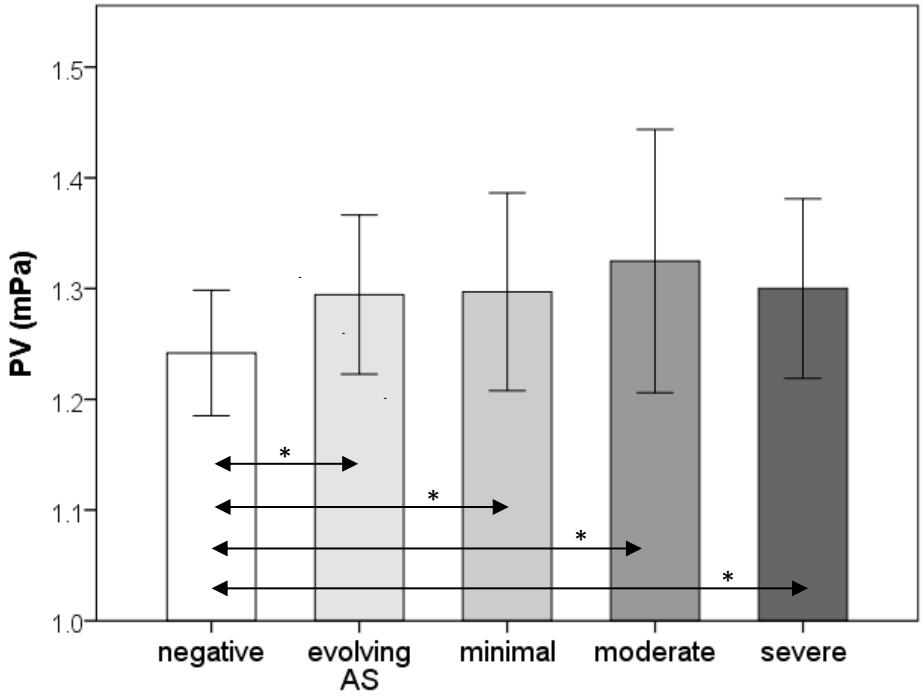


*Figure 10: Differences in plasma viscosity among CAS groups. Values are means ± SD. *p<0.05*

In current smokers Hct and WBV (at all shear rates) were significantly higher than in current non-smokers. These differences were detected only between the never smoking group and the present smoking group. In the symptomatic subgroup Hct and WBV were significantly elevated, and interestingly red blood cell deformability (EI at 1.69 -0.3 Pa and $SS_{1/2}$) was impaired in non-smokers. In the asymptomatic group besides increased Hct and PV, deteriorated red blood cell aggregation was observed in current smokers. In the stenotic group we found Hct, PV, and WBV (at all shear rates) higher in smokers. There were no differences in the non-stenotic group. (Table 3)

n		Hct (%)	WBV at 90 s^{-1} (mPas)	PV (mPas)	AI	$SS_{1/2}$ (Pa)
All patients	Non-smokers (83)	42.8±4.2	4.05±0.4	1.29±0.10	67.76±5.88	1.89±0.3
	Smokers (16)	46.8±5.5 **	4.61±0.7 **	1.33±0.10	70.15±5.62	1.77±0.2
Asymptomatic	Non-smokers (60)	42.8±4.2	3.98±0.4	1.28±0.06	67.78±4.8	1.81±0.3
	Smokers (5)	48.6 ±7.6**	4.40±0.8	1.35±0.15 *	73.39±6.06 *	1.78±0.4
Symptomatic	Non-smokers (31)	42.7±4.4	4.22±0.4	1.33±0.13	67.74±7.37	2.00±0.2
	Smokers (11)	46.0±4.5 *	4.68±0.7 *	1.32±0.08	68.67±5.00	1.76±0.1**
Non-stenotic	Non-smokers (48)	42.4±4.4	4.0±0.4	1.29±0.87	66.85±5.04	1.86±0.3
	Smokers (7)	44.3±3.5	4.1±0.3	1.26±0.05	67.80±3.85	1.69±0.2
Stenotic	Non-smokers (43)	43.2±4.1	4.1±0.3	1.30±0.10	68.66±6.54	1.92±0.3
	Smokers (9)	48.8±6.1 **	5.1±0.7**	1.38±0.10 **	71.98±6.28	1.83±0.1

Table 3: Differences between non-smokers and smokers.

*Values are means ± SD. *p<0.05, **p<0.01*

4.4. Discussion

Several researches have investigated the connection between hemorheological parameters and stenosis of carotid arteries, but methods of hemorheological measurements and patient classification according to stenosis were different. We decided to compare clinically significant stenosis to non–significant stenosis.

Previous investigations have described the changes in hemorheology in stenosis among chronic cerebrovascular patients: increased hematocrit [52, 54, 56, 62] was found; whole blood viscosity, plasma viscosity [52, 56, 61], and red blood cell aggregation were also elevated [52, 53]. In acute stroke no differences were found in hematocrit and WBV either, but plasma viscosity was higher in patients who had severe CAS as well [63]. Our results are similar to these findings although we investigated a mixed population, not only symptomatic patients.

We observed reduced red blood cell deformability, elevated whole blood and plasma viscosity in symptomatic patients compared to asymptomatic patients. Others found higher hematocrit, fibrinogen, and RBC aggregation as well, not visible in our results. However, our asymptomatic patients were age-matched controls who had cardiovascular risk factors, while controls in these studies were younger healthy volunteers [52, 56].

Evaluation of the effect of stenosis and cerebrovascular event in history based on subgroup analysis suggests that while PV is altered only with symptoms and Hct only with stenosis, changes in WBV and red blood cell deformability can be signs of both of them. However, it is still unclear whether altered hemorheology is a consequence of cerebrovascular events or the presence of deteriorated hemorheological parameters can lead to stroke or TIA, which remain worsened after the events.

Further subgroup analysis demonstrated no correlation between rheology and degree of stenosis, severe and moderate stenosis could not be separated by these factors. Nevertheless, plasma viscosity and red blood cell deformability were worse than in the negative group not just in CAS groups but in evolving atherosclerosis as well, which can imply the possible role of these factors in atherosclerosis formation. Prior findings proposed red blood cell deformability and fibrinogen level as potential markers of atherosclerotic plaque formation in patients 3 months after acute stroke [61] and suggested that plasma fibrinogen concentration may predict the progression of stenosis [60, 4]. Other studies found correlation between hematocrit and intima-media thickness [58, 4], and between plasma viscosity and early phase atherosclerosis [55, 4], while another investigation indicated IMT related plasma viscosity and reduced red blood cell filterability having minor relevance, and explained differences in fibrinogen level by smoking [55].

Smoking causes hemorheological disturbances like increased Hct, WBV, PV, fibrinogen, deteriorated red blood cell aggregation, and deformability [4, 5, 52, 64]. Our results suggest that present smoking is a relevant factor in hemorheology, but smoking history does not have a significant role despite a recent finding implying that past smoking has a relevant effect, too [64]. Smoking worsened Hct and whole blood viscosity, furthermore plasma viscosity was higher in the asymptomatic and stenotic subgroup, and red blood cell aggregation was increased only in asymptomatic patients. Surprisingly, red blood cell deformability was deteriorated in non-smokers among symptomatic patients. Only age can explain this discrepancy: non-smokers were significantly ($p=0.006$) older than smokers (non-smokers 64.6±6 years vs. smokers 59.0±4 years), while there were differences neither in sex, stenosis, chronic diseases, nor in drug therapy.

In routine laboratory examinations cholesterol and LDL were significantly higher in asymptomatic patients than in symptomatic patients and also in the non-stenotic group compared to the stenotic group; it can be explained by the fact that most of the non-stenotic patients with no history of cerebrovascular event were poorly medicalized receiving lipid lowering agents in about 50%. Statin therapy can affect microrheological properties, but not macrorheology [65]; thus our results of RBC aggregation and deformability could have been more pronounced if our patient groups had recieved the same therapy.

Our research indicates that hemorheological parameters could be affected by stenotic carotid artery, furthermore clinically significant stenosis and the history of a cerebrovascular event themselves have a remarkable role. Even though we suppose that these factors cannot be suitable markers, presence of atherosclerosis may be detected.

5. The relationship between hemorheological parameters and mortality in critically ill patients

5.1. Introduction

Mortality estimation in intensive care unit (ICU) and scores

Measuring mortality predictions and illness severity has become increasingly important in critical care medicine. Prognostic models are used for assessment of quality of care and to help the physicians in treatment options and to allocate the limited ICU resources. They can also help to evaluate the effect of newer therapies and interventions, and organizational changes. Furthermore, these scoring systems are essential in randomised control trials and clinical researches for risk stratification and to prove the equivalency of study and control patients [66, 67].

After the first prognostic model, the Therapeutic Intervention Scoring System was introduced in 1974; severity scoring systems have been developed during the last three decades. Several prognostic scores exist, but the two most commonly used adult-ICU prognostic scoring systems are Acute Physiology and Chronic Health Evaluation (APACHE) and Simplified Acute Physiology Score (SAPS) [66, 67].

APACHE was created in 1978 based on Acute Physiology, age and chronic health state from collected information of ICU patients. It was followed by APACHE II in 1984, which is, next to SAPS II, the most commonly used model worldwide. It used 12 physiological values from the first 24 hours after admission and added 56 disease groups to the first score. Outcome prediction was based on data of US hospitals from 1979-1981. However, it has become obsolete with the appearance of new therapies and the change of co-morbidities. Therefore,

APACHE III was developed, which extended the number of patient information and disease groups, and it could predict not only mortality, but also the length of stay. APACHE IV was described in 2006. The main component in the APACHE IV predictive equations was APACHE III score, but it was based on data from 2002-2003 [68-71].

SAPS was designed in 1984 by ICUs of Western Europe to simplify the existing APS or Apache score. It included 14 variables and the following SAPS II in 1993 needed only 17 variables that could contribute to its spread. The new SAPS III showed some novelty in 2005. This score is based on data collected within the 1st hour of ICU admission therefore, it could predict outcome before ICU intervention occured [72, 73].

Nevertheless, the utility of scores are still controversial. It is reported that Apache II and SAPS II tend to underestimate hospital death in patients with lower mortality probability. APACHE IV has good discrimination and accuracy, but the calibration is poor, in addition, it is very time consuming. Moreover, although they perform well in the general ICU population, they cannot predict the mortality of individual patients [74, 75].

Hemorheological aspects of critically ill patients

Microcirculation has a crucial role in oxygen delivery and maintenance of tissue perfusion. It may be a reason why multiple organ failure (MOF) can develop in spite of the correction of global hemodynamic parameters. Sepsis is characterised by profoundly disturbed microcirculation with the decrease in the density of functioning capillaries, increase in non-perfused and intermittently perfused vessels and functional shunting. Prior researches have indicated that these alterations can have a prognostic value in septic shock [76-78]. Hemorheological properties, which are important factors of microcirculation, can be essential in critical conditions, especially in sepsis.

Previous publications investigated hemorheological parameters mainly in sepsis. They suggested that red blood cell deformability is reduced in sepsis and it can be a marker of the severity of sepsis, furthermore it can refer to impaired oxygen utilization and multiple organ damage [79-90]. Red blood cell aggregation is increased in sepsis and it correlates with prognostic scoring systems [86, 89, 91, 92]. Macrorheological factors, like whole blood viscosity (WBV) and plasma viscosity (PV) can be altered in septic and also in nonseptic patients [92]. Only one recent study researched these variables in correlation with outcome and highlighted the potential effect of microrheology [90].

Estimating mortality risk in intensive care unit is complicated although several mortality scoring systems have been developed in the past decades. The conventional systems (Apache II and SAPS II) use basic hemodynamic, laboratory (electrolytes, kidney function tests, arterial blood gases, white blood cell count), Glasgow Coma Scale and some anamnestic data, while in novel scoring systems the admission diagnoses and medical history are more pronounced. In spite of these steps of evolution only few new laboratory parameters were added (glucose and albumin to Apache IV and platelet count to SAPS III), but except for hematocrit in Apache systems there are no hemorheological markers in any of the scoring systems [66-73].

Nevertheless, the role of hemorheological parameters among critical conditions in a heterogeneous intensive care population and the possibility of them being prognostic markers remained unclear. This report describes the relationship among hemorheological parameters, mortality and clinical outcome in a heterogeneous population in an Intensive Care Unit.

5.2. Methods

Patients and study design

112 patients treated in intensive care unit (ICU) with different non-surgical diseases were recruited. Exclusion criteria were age under 18 years, admission from another ICU, and ICU readmission. Blood samples were drawn from patients within the first 24 hours after ICU admission. Blood sampling for hemorheological parameters was performed on the 2nd day in 83 patients as well (others deceased or were discharged to other non-intensive care department), and the change of values (Δ) was calculated. At ICU admission the presence of sepsis (identified using standard criteria as the presence of infection together with evidence of a systemic inflammatory response syndrome) and diagnosis were recorded, and ICU mortality scores (Acute Physiology and Chronic Health Evaluation (APACHE) II and IV score, Simplified Acute Physiology Score (SAPS) II and III [66-74]) were calculated (Table 4).

Hemodynamic parameters and received therapy in ICU were assessed. There was no significant difference between septic and nonseptic patients regarding age and admission diagnoses, although acute renal failure and hematological disorders were more common in the septic group. In therapy mechanical ventilation (41.3% in nonseptic, 60.6% in septic), vasopressor (52.2% nonseptic, 81.8% in septic), and antibiotics (28.3% in nonseptic, 75.8% in septic) were more frequent in septic patients.

Mortality was followed up to 30 days. ICU mortality was 37.5%, while 30-day mortality was 46.6%.

Laboratory measurements

Arterial blood was collected into K_2EDTA coated Vacutainer tubes. Blood samples were taken from the patients' arterial catheter. Routine laboratory (electrolytes, osmolality, renal function tests, liver function tests, coagulation markers, fibrinogen, total protein, albumin, C-Reactive Protein, procalcitonin, arterial blood gases, and complete blood count) parameters were determined. Hemorheological measurements were carried out within 2 hours after blood sampling [2, 22-24], according to the above mentioned methodological description (see page 10-13).

	total	**ICU mortality**		**30-day mortality**	
		survivors	**nonsurvivors**	**survivors**	**nonsurvivors**
n	112	70	42	60	52
age	67.8±12	67.6±13	68.0±11	66.4±13	69.4±12
male/female	58/54	35/35	23/19	32/28	26/26
length of stay (day)	7.2±10	6.2±9	9.0±11	8.3±12	5.9±5
Apache II mortality (%)	39.7±27	31.2±24	54.0±25 **	27.2±21	54.2±26 **
Apache IV mortality (%)	35.2±29	24.8±24	52.5±28 **	20.2±21	52.5±27 **
Apache IV LOS (day)	5.1±2	4.5±2	6.2±2 **	4.4±2	5.9±2 **
SAPS II mortality (%)	41.1±31	33.2±29	54.3±31 **	28.7±27	55.5±30 **
SAPS III mortality (%)	41.3±25	33.1±22	54.8±25 **	29.7±20	54.6±25 **
sepsis at admission	66 (59%)	35 (53%)	31 (74%) **	28 (42%)	38 (58%) **
respiratory failure	77 (68%)	41 (59%)	36 (86%) **	35 (58%)	42 (81%) **
heart failure	47 (42%)	29 (41%)	18 (43%)	22 (37%)	25 (48%)
acute renal failure	50 (45%)	25 (36%)	25 (59%) **	19 (32%)	31 (59%) **
hematological disorder	11 (10%)	3 (4%)	8 (19%) *	2 (3%)	9 (17%) *

*Table 4: Demographic characteristics, ICU score points, and diagnosis at admission, and therapy of patients. *p<0.05,* **p≤0.01

Ethical aspects

The study was approved by the Regional Ethics Committee of the University of Pecs and an informed consent was signed by all subjects or their relatives (number of approval: 5828).

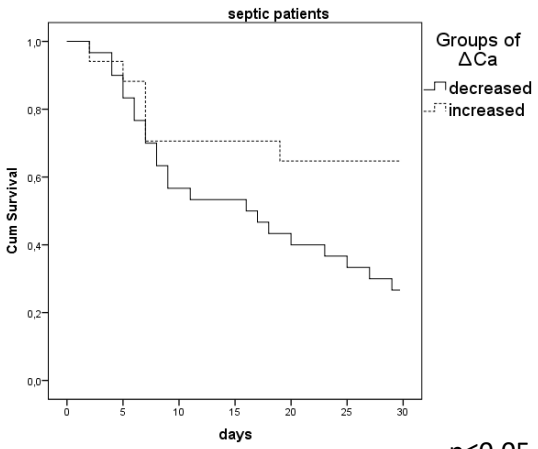
Statistical analysis

IBM SPSS statistical software version 22 was used. Data are expressed as means ± SD. Variables that are considered normally distributed with Shapiro-Wilk test were evaluated by Independent sample T-test. Nonparametric Mann-Whitney U-test was applied for non-normally distributed variables. For survival analysis Kaplan Meier test and Cox proportional hazard model was used. Significance level was defined as $p < 0.05$.

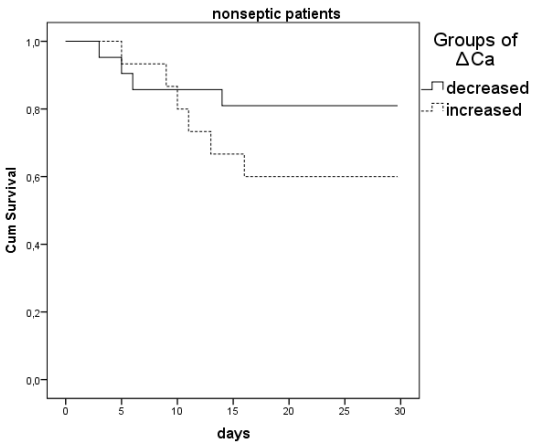
5.3. Results

Table 5 shows hemodynamic and laboratory parameters of survivors and nonsurvivors. Heart rate was higher, blood pressure was lower in nonsurvivors. Ca level was decreased in nonsurvivors and osmolality was increased in patients who did not survive 30 days, and in septic patients. Kaplan-Meier analysis and Cox proportional hazard model represented increased changes in Ca level with about twice higher mortality risk (Hazard ratio=2.587, CI: 1.045-6.408) and patients with Δosmolality above the median have about 3-times lower survival rate (HR= 2.986, CI: 1.306-6.827, Figure 11).

a, *ΔCa in septic patients*

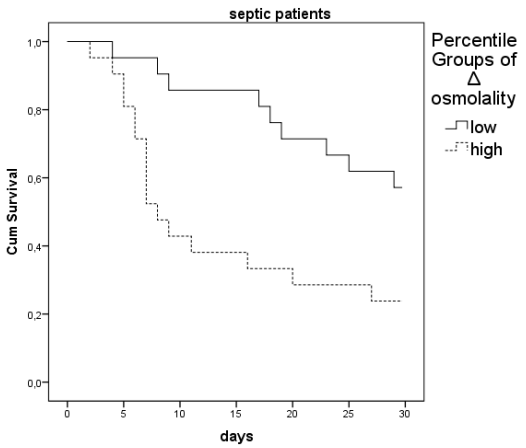


b*, ΔCa in nonseptic patients*

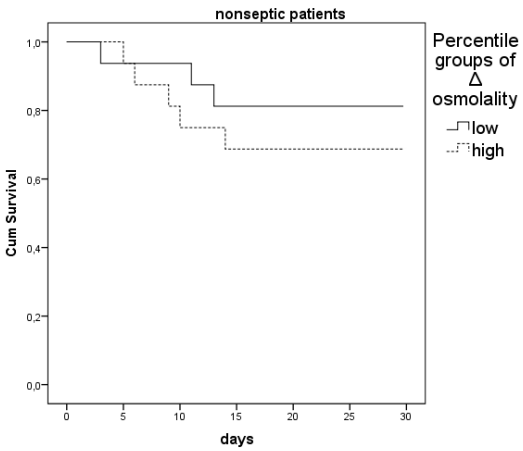


p≤0.05

d, *Δ osmolality in septic patients*



c, *Δ osmolality in nonseptic patients*



p≤0.05

Figure 11: Survival (Kaplan-Meier) curves of septic and *nonseptic patients (a,* ΔCa, b, Δ osmolality).

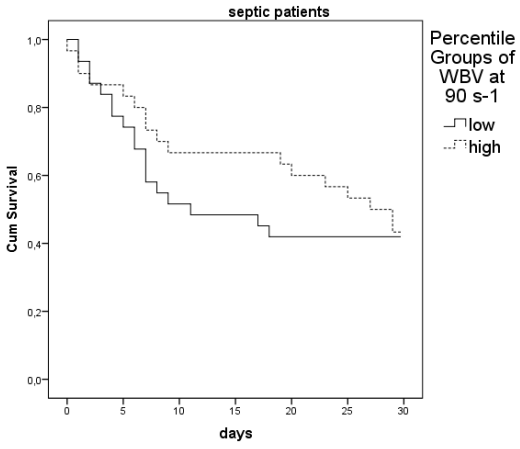
	ICU mortality		30-day mortality	
	survivors	**nonsurvivors**	**survivors**	**nonsurvivors**
Heart rate (beats /minute)	88±20	97±26 *	87±21	98±23 *
SBP (mmHg)	123±16	114±23 *	123±16	115±22 *
MAP (mmHg)	82±13	75±13 **	81±13	77±14
urine output (ml/24 h)	2514±1488	2024±1345	2572±1405	2045±1459
Na (mmol/l)	138.1±7	137.6±5	137.8±7	137.9±5
K (mmol/l)	4.37±0.7	4.34±0.6	4.37±0.7	4.34±0.6
Ca (mmol/l)	1.99±0.2	1.88±0.2 **	1.98±0.2	1.91±0.2 *
osmolality	292.6±13	295.8±14	290.8±12	297.2 ±14*
creatinine (mmol/l)	168.6±164	187.9±161	173.3±173	178±143
glucose (mmol/l)	8.6±4	7.8±3	8.6±4	8.0±3
bilirubin (umol/l)	17.4±20	27.2±34	17.1±21	25.6±31
GOT (U/l)	255.8±691	416.9±1476	210.8±703	384.1±1352
total protein (g/l)	55.4±8	51.2±10 *	54.9±8	52.7±10
albumin (g/l)	31.1±6	25.7±6 **	31.3±5	26.4±6 **
CRP (mg/l)	90.1±97	136.4±111 **	95.7±109	120.9±98
PCT (ng/ml)	5.5±15	13.2±27 **	7.4±20	9.6±22
WBC (Giga/l)	11.9±6.3	19.8±18.9 *	12.0±6	18.2±17 *
Hct (%)	33.25±7.7	33.1±6.9	33.4±8	32.9±6
platelet (Giga/l)	205.8±98	195.1±144	197.5±86	206.5±146
INR	1.53±0.6	1.90±1.2 *	1.52±0.6	1.84±1.1
fibrinogen (g/l)	3.99±1.7	4.13±1.9	3.95±1.7	4.16±1.8
pH	7.4±0.08	7.3±0.12 **	7.4±0.07	7.34±0.12 **
pCO2 (mmHg)	40.7±13	40.0±13	40.7±13	40.2±13
pO2 (mmHg)	96.7±39	97.6±42	92.1±29	102.8±49
HCO3 (mEq/l)	25.2±6	21.9±6 *	25.2±5	22.6±7 *
lactate (mmol/l)	1.98±2.1	3.55±3.9 *	1.82±1.9	3.43±3.7 **
mechanical ventilation (h)	39.5±49	116.6±56 **	52.8±123	82.8±106
total vasopressor (h)	33.8±49	66.0±56 **	31.7±48	60.9±56 **

*Table 5: Differences between laboratory measurements and between the received therapies. Values are means ± SD. *p<0.05, **p<0.01*

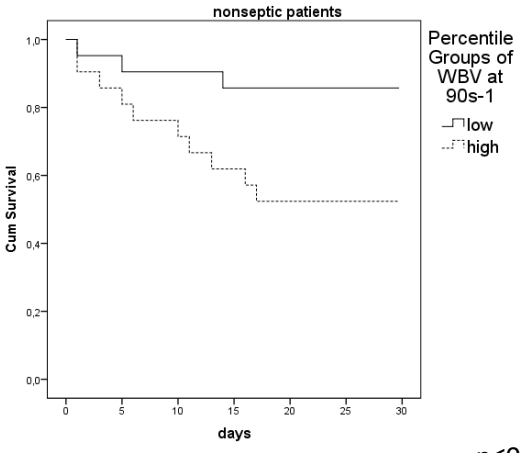
Total protein and albumin level were lower in nonsurvivors. INR was increased in patients who died in the ICU. Inflammatory parameters were detected higher and nonsurvivors were more prone to metabolic acidosis and elevation of lactate level. Fibrinogen was not different between survivors and nonsurvivors, but its 1st –day level was higher in septic than in nonseptic patients, while the change between the measurements was higher in nonseptic patients (Table 13).

Table 6 describes hemorheological parameters. There was no difference in capillary hematocrit level between survivors and nonsurvivors. The 1st-day measurements showed no differences among the groups, but the 2nd-day WBV was higher in nonsurvivors among nonseptic patients. No differences could be detected in PV values, nevertheless an increasing tendency from the 1st to the 2nd measurement was found in nonsurvivors. In nonseptic patients Kaplan-Meier analysis showed that patients with WBV values above the median had poorer survival (Figure 12), with about 4-times higher mortality risk according to Cox proportional hazard model (HR of 1st-day WBV at 90 s^{-1} = 3.968, CI: 1.090-14.453).

a, *WBV in septic patients*



b, *WBV in nonseptic patients*



p≤0.05

Figure 12: Survival (Kaplan-Meier) curves of whole blood viscosity in septic (*a*) and *nonseptic patients* (*b*).

		Total		nonseptic		septic	
		survivors	nonsurvivors	survivors	nonsurvivors	survivors	nonsurvivors
Hct (%)	1st day	36.4±8	34.9±8	35.5±9	39.0±6	37.2±8	33.5±8
	2nd day	36.3±7	36.7±8	35.4±7	40.7±7	37.1±8	35.3±8
	Δ	-0.9±4	1.1±4	1.3±4	-0.1±3	-1.6±4	1.6±5
PV (mPas)	1st day	1.22±0.1	1.24±0.1	1.20±0.1	1.25±0.1	1.24±0.1	1.23±0.1
	2nd day	1.21±0.1	1.26±0.1	1.19±0.1	1.26±0.1	1.20±0.1	1.26±0.2
	Δ	-0.01±0.1	0.03±0.10*	-0.00±0.1	0.03±0.1	-0.16±0.3	0.02±0.4 *
WBV at 400 s^{-1} (mPas)	1st day	2.69±0.6	2.71±0.5	2.65±0.6	2.92±0.4	2.72±0.5	2.62±0.4
	2nd day	2.62±0.4	2.79±0.6	2.61±0.4	3.29±0.4 **	2.62±0.4	2.59±0.5
	Δ	-0.04±0.3	0.10±0.4	0.05±0.3	0.26±0.4	-0.04±0.7	0.03±0.1
WBV at 90 s^{-1} (mPas)	1st day	3.28±0.7	3.27±0.6	3.24±0.8	3.54±0.6	3.31±0.6	3.17±0.6
	2nd day	3.23±0.6	3.36±0.8	3.25±0.7	3.98±0.6 *	3.17±0.4	3.11±0.7
	Δ	-0.02±0.6	0.09±0.6	0.13±0.7	0.3±0.5	-0.21±0.5	0.00±0.6
AI	1st day	66.3±10	67.33±10	63.2±10	69.8±9 *	69.4±10	66.4±11
	2nd day	67.7±7	66.1±8	66.1±8	74.9±5 *	69.4±9	66.4±11
	Δ	1.3±7	0.9±5	3.4±7	2.5±3	-1.1±5	0.3±5
t ½ (sec)	1st day	2.02±1.4	2.00±1.7	2.34±1.4	1.60±0.9 *	1.69±1.3	2.15±1.8
	2nd day	1.79±1.0	1.63±1.1	1.93±0.9	1.12 ±0.4 **	1.66±1.0	1.81±1.3
	Δ	-0.25±1.0	-0.28±0.9	-0.52±1.1	-0.20±0.2	0.09±0.6	-0.30±1.1
γ (s^{-1})	1st day	200.7±83	207.9±92	191.0±85	183.2±85	212.9±80	217.2±91
	2nd day	208.2±97	233.7±88	206.3±106	199.7±81	213.2±88	246.1±88
	Δ	-9.7±64	44.9±106 *	10.2±76	24.1±58	9.5±53	52.5±0.119
EI at 30 Pa	1st day	0.599±0.02	0.594±0.03	0.600±0.02	0.608±0.02	0.597±0.02	0.588±0.03
	2nd day	0.602±0.02	0.591±0.02 *	0.607±0.02	0.606±0.01	0.595±0.02	0.586±0.02
	Δ	0.004±0.02	-0.004±0.02*	0.007±0.02	-0.004±0.02	0.000±0.02	-0.004±0.1
EI at 3 Pa	1st day	0.398±0.02	0.389±0.03	0.400±0.02	0.409±0.02	0.395±0.02	0.382±0.03
	2nd day	0.405±0.02	0.393 ±0.03*	0.407±0.02	0.410±0.02	0.400±0.02	0.386±0.03
	Δ	0.006±0.01	0.003±0.03	0.007±0.02	-0.003±0.01	0.004±0.01	0.001±0.03

Table 6: Differences between hemorheological measurements.
*Values are means ± SD. *p<0.05, **p<0.01*

Among nonseptic patients, nonsurvivors showed increased RBC aggregation in the first day (Table 6). Kaplan-Meier analysis showed a significant difference in 30-day survival of patients with aggregation above or below the median in the total population (t½ HR =0.570 CI: 0.325-1.000). It was more prominent in the nonseptic group (Figure 13) with about 4-times higher Hazard ratio for higher aggregation (HR =4.413, CI: 1.228-15.858), although in the septic group no difference was found.

a, *AI in septic patients* b, *AI in nonseptic patients*

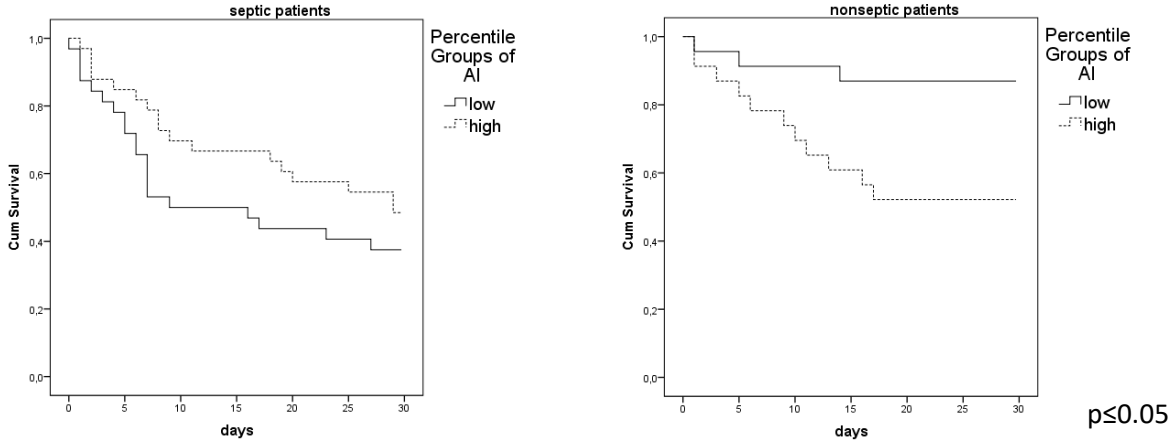
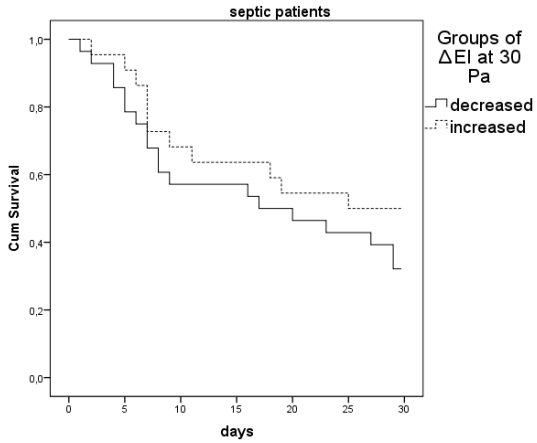


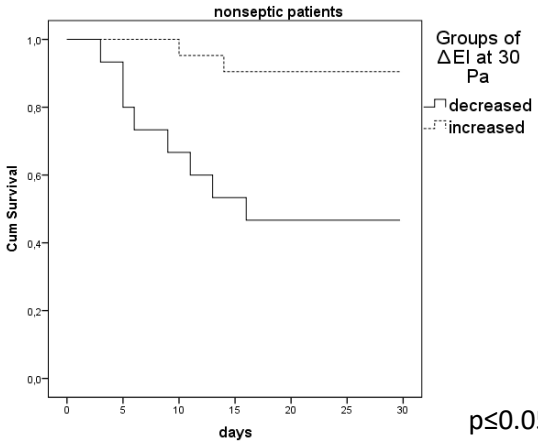
Figure 13: Survival (Kaplan-Meier) curves of red blood cell aggregation in septic (*a*) and *nonseptic patients* (*b*).

In nonsurvivors RBC deformability (at higher shear stresses) showed worsening from the 1st to the 2nd day, and it was found to be lower on the second day compared to survivors (EI at 30-3 Pa, Table 6) in the whole examined population. Although survival analysis referred to lower survival in patients whose RBC deformability worsened from the 1st to the 2nd day compared to those whose RBC deformability improved (HR of ΔEI at 30 Pa=2.669, CI:1.375-5.181), no relationship was found in sepsis during subgroup analysis. In nonseptic patients survival analysis represented 7-times higher mortality risk in patients with worsened RBC deformability (Figure 14, HR of ΔEI at 30 Pa=7.647, CI: 1.617-36.173).

a, *ΔEI in septic patients*



b, *ΔEI in nonseptic patients*



p≤0.05

Figure 14: Survival (Kaplan-Meier) curves of red blood cell aggregation in septic (*a*) and *nonseptic patients* (*b*).

1st day fibrinogen was higher in sepsis and it decreased more than in nonseptic patients. Although the 1st day WBV and RBC aggregation were not different between septic and nonseptic patients, changes of WBV between the 1st and 2nd day decreased in sepsis, and changes of RBC aggregation increased in nonseptic patients. RBC deformability (EI at 30-1.69 Pa) was impaired in septic patients both on the 1st and on the 2nd day (Table 7).

		nonseptic	septic
Hct (%)	1st day	36.3±8	35.4±8
	2nd day	36.5±7	36.2±8
	Δ	0.9±4	-0.1±4
fibrinogen (g/l)	1st day	3.48±1.3	4.44±2.0 **
	2nd day	3.84±1.3	4.23±2.0
	Δ	0.57±0.9	-0.02±0.7 **
PV (mPas)	1st day	1.21±0.1	1.23±0.1
	2nd day	1.21±0.1	1.24±0.1
	Δ	0.005±0.08	-0.006±0.12
WBV at 400 s^{-1} (mPas)	1st day	2.72±0.6	2.67±0.5
	2nd day	2.76±0.5	2.61±0.4
	Δ	0.11±0.3	-0.07±0.4 *
WBV at 90 s^{-1} (mPas)	1st day	3.31±0.7	3.24±0.6
	2nd day	3.42±0.7	3.14±0.6
	Δ	0.17±0.6	-1.1±0.5 *
AI	1st day	64.8±10	68.0±11
	2nd day	73.9±32	68.5±9
	Δ	8.9±35	1.1±13
t ½ (sec)	1st day	2.16±1.3	1.91±1.5
	2nd day	1.75±0.9	1.74±1.1
	Δ	-0.45±1.0	0.10±0.9 *
γ (s^{-1})	1st day	189.1±86	214.9±84
	2nd day	204.8±100	229.2±88
	Δ	13.4±71	30.1±92
EI at 30 Pa	1st day	0.602±0.02	0.592±0.02 *
	2nd day	0.607±0.01	0.591±0.02 **
	Δ	0.005±0.02	0.003±0.02
EI at 3 Pa	1st day	0.403±0.02	0.389±0.03 *
	2nd day	0.408±0.02	0.393±0.03 **
	Δ	0.005±0.02	0.003±0.02

Table 7: Differences between nonseptic and septic patients.
*Values are means ± SD. *p<0.05, **p<0.01*

Red blood cell aggregation correlated significantly with prognostic scores (1st-day AI, $r_{Apache\ IV}$ =0.213; $r_{Apache\ IV\ LOS}$ = 0.277; $r_{SAPS\ II}$ =0.185; $r_{SAPS\ III}$ =0.187). Correlation with red blood cell deformability was significant with 2nd-day EI at 30Pa ($r_{Apache\ II}$ =-0.247; $r_{SAPS\ III}$ =-0.312), as well as with Δ EI at 30Pa ($r_{Apache\ IV}$ =-0.285, $r_{Apache\ IV\ LOS}$ =-0.282, $r_{SAPS\ II}$ =-0.226, $r_{SAPS\ III}$ =-0.215). There was no relationship between ICU scores and macrorheological parameters, but fibrinogen level correlated significantly with scores ($r_{Apache\ II}$=-0.213, $r_{Apache\ IV}$ =-0.224, $r_{Apache\ IV\ LOS}$ =-0.329, $r_{SAPS\ II}$ =-0.206).

To evaluate if hemorheological parameters could provide further information about mortality risk to ICU scores, dichotomised hemorheological parameters (AI, WBV- being lower or higher than the median; ΔEI at 30Pa- positive or negative) as categorical variables were added to ICU scores in Cox proportional hazard models. In septic patients none of these parameters remained significant. In nonseptic patients higher AI, higher WBV and negative ΔEI meant increased mortality risk in the various models (Hazard ratios [CI]: $AI_{APACHE\ II}$=4.151 [1.144-15.066], $WBV_{APACHE\ II}$=5.047 [1.247-18.912], $\Delta EI_{APACHE\ II}$ =10.329 [2.020-52.829]; $WBV_{APACHE\ IV}$ =3.981 [1.070-14.807], $\Delta EI_{APACHE\ IV}$ =6.986 [1.457-33.487]; $\Delta EI_{SAPS\ II}$ =7.017 [1.477-33.334]; $\Delta EI_{SAPS\ III}$ =6.060 [1.257-29.209]).

5.4. Discussion

Several researches investigated hemorheological parameters in critically ill patients in the past few decades. Deteriorated red blood cell deformability in sepsis was described in animal models [79, 82, 83] and also in patients [79-81, 83, 84, 87-90, 92]. These studies suggested that decreased deformability can be a consequence of reactive oxygen superoxide, 2.3 diphosphoglycerate increase or the presence of lipopolysaccharide, and it can be a marker of the severity of sepsis. Increased RBC aggregation in sepsis [89, 91, 92] and altered macrorheological parameters [87, 91, 92] in critically ill patients are also known. However, only a few studies can be found in correlation with survival [90, 92] or ICU scoring systems [87-89, 92].

In routine laboratory examination we found inflammatory parameters higher, albumin and total protein lower in nonsurvivors than in survivors, and the deterioration of ABG parameters were more characteristic in nonsurvivors. Interestingly, sodium, potassium, glucose, renal function parameters, bilirubin, and Hct were not different in spite of the fact that they are main parts of ICU scores. Other surprising results were the lower Ca, INR and the higher osmolality in nonsurvivors that are not in any scores, furthermore the change of Ca and osmolality could refer to survival. Although hypocalcaemia is common in Intensive Care unit, it is controversial if it is associated with the outcome [93]. Recent data confirm our findings with decreased prothrombin INR suggesting that it can be a predictive marker [94] and a previous investigation found association between osmolality and outcome in patients with acute coronary syndrome [95].

Evaluation of macrorheology remained in question. In whole blood viscosity a decreasing tendency could be detected in septic patients, while an increasing tendency in nonseptic

patients, but no differences were found in plasma viscosity values. A previous study has described a similar result in WBV, but they found higher PV in sepsis [92]. Others have not found WBV different, but PV was lower in sepsis [84]. In spite of these differences in WBV, in our research it had no relationship to survival in all patients, but subgroup analysis showed a significant connection in nonseptic patients. Hyperviscosity reduces blood fluidity and causes microcirculatory failure [1], but in sepsis hyperkinetic circulation and slowly progressing anemia may prevent hyperviscosity syndrome [96]. It can explain that blood viscosity has a role only in nonseptic patients. Fibrinogen was elevated in sepsis and its increasing tendency was parallel with the elevation from day-1 to day-2 of red blood cell aggregation in nonseptic patients, but it affected the outcome neither in septic nor in nonseptic patients.

Red blood cell aggregation was increased in nonsurvivors, among nonseptic patients, and it correlated with ICU scores, suggesting that red blood cell aggregation can have a significant role in this patient group. Although no differences could be detected between septic and nonseptic patients, interestingly in nonseptic patients t ½ shortened more over time than in septic patients, referring to a higher aggregation ability. In contrast, previous studies described that no relationship was found with outcome [92], but an association with sepsis and scores [89].

Several previous researches have explored impaired red blood cell deformability in sepsis and also revealed it as a marker of severity [88], it was lower than in nonseptic patients at higher shear stresses as our study indicated [89, 90]. Only one recent finding has reported a link between the worsening of deformability and mortality, even though they found it only in sepsis [90]. We observed a strong relationship between the deterioration of deformability and outcome in nonseptic patients, but not in sepsis. It can imply that in sepsis, where

deformability is originally lower, further reduction does not have more serious consequences, but in nonseptic patients worsening can refer to the decreasing microcirculatory functions. Other explanation can be that in sepsis the profound microcirculatory alterations, increased permeability and capillary diameter, and the elevated vascular tone could hide the effect of deformability. Change of deformability might reflect the response to therapy or the capability to recovery. Patients, who could not maintain or increase the ability of red blood cells to deform, had higher risk to mortality.

Presence of sepsis has an explicit effect on survival and it is also a major component of ICU scores. Nevertheless, sepsis is largely different from nonseptic condition; there may be markers that refer to mortality only in septic or nonseptic patients. Hemorheological parameters had a dissimilar behaviour in septic and nonseptic patients and our results suggested that whole blood viscosity and red blood cell aggregation of nonseptic patients could be informative about ICU scores. The deterioration of red blood cell deformability may also add details to understanding mortality risk, although it is a dynamic parameter and data from the 2nd day are also needed, whereas scores use static variables from the 1st day. These parameters could be considered to be researched in a multicenter investigation.

The present study has some limitations. Firstly, it would provide more information if we performed measurements immediately after admission. Viscosity is affected by fluid intake; the different periods of time from admission until blood collection, the different amount of received infusion may limit our results. However, hemorheological parameters should be measured within 2 hours after blood sampling and it is not available any time during the day. Furthermore, it is questionable how to define the optimal time: earlier sampling will be inaccurate because of the therapy taken before the intensive care admission, later

measurements will be affected by ICU therapies. Secondly, receiving transfusion can affect blood rheology. We decided to investigate a heterogeneous population including transfused patients. Our hypothesis was that survival depends on the properties of circulating blood, even if it is own blood or received. Thirdly, the relatively small sample size can limit the value of our observations and explanations. It can explain that hemorheological parameters had to be dichotomised, because survival analysis had poor results with continuous variables. A multicenter study is needed to confirm our findings and it could have clinical implications. Fourthly, these special parameters are not available in every laboratory and they are useful in one certain patient group, therefore it could only be optional in clinical practice.

In conclusion, our research suggested that Ca and osmolality can predict mortality in septic patients, and whole blood viscosity, red blood cell aggregation and the change of red blood cell deformability in nonseptic patients. Further investigations of microcirculatory alterations can help to understand pathophysiology of critical conditions and multicenter researches could evaluate the role of these parameters in estimating mortality risk.

6. Conclusion

Researches in the field of flow properties of blood and blood cells have developed in the past decades. Many aspects of hemorheology can be affected by diseases or clinical states therefore it can be relevant in clinical practise [1]. We believe that our results could contribute to broadening our knowledge of disturbed blood rheology.

Risk factors and pathomechanism of atherosclerosis have been investigated in the past decades. These findings suggested that impaired blood flow further accelerates plaque formation that refers to the potential role of hemorheological factors. Increased hematocrit and RBC aggregation have been described, but our results confirmed those that found plasma viscosity and RBC deformability as a marker of atherosclerotic lesion. These factors can be not only signs, but they can also be involved in the pathomechanism. Due to the film layer formation in the capillary lumen, plasma viscosity has a direct effect on the endothelial surface; on the other hand, it can contribute to the impairment of microcirculation and also to the increase in blood flow resistance [1, 97]. Deformability can also affect the endothelial wall owing to the increase of blood viscosity and the subsequent increase in shear stress. Although our findings did not prove the association between these parameters and the progression of atherosclerosis, they can indicate its presence.

The effect of hemorheological properties on outcome in critically ill patients is not well researched. Although it is well known that sepsis is characterised by microcirculatory alterations and in these disturbances impaired hemorheological factors can be involved [76-89], only one recent study investigated their potential prognostic role [90]. Our results

indicated that whole blood viscosity, RBC aggregation and deformability could refer to survival, although we found it only in nonseptic patients in contrast to those researches that found it in sepsis. A multicenter study could confirm our findings and clarify which population could profit from these results. However, the mechanisms behind these alterations are still unclear. Further investigations could reveal the molecular processes that lead to the difference between survivors and nonsurvivors and they could help to understand the importance of these factors.

7. Summary of the new scientific results

1. Hemorheological alterations in carotid artery stenosis

1, We confirmed the impairment of hemorheological parameters in case of carotid atherosclerosis, but we did not find relationship between the magnitude of this impairment and the magnitude of carotid stenosis.

2, We demonstrated that whole blood viscosity, plasma viscosity and red blood cell deformability are worse in patients with previous cerebrovascular event even compared to age-matched controls and not only to healthy young volunteers as in previous publications.

3, We showed that smoking can further deteriorate rheological properties of the blood even in the advanced stage of carotid atherosclerosis; when significant stenosis is already present or the patient had a previous cerebrovascular event.

2. The relationship between hemorheological parameters and mortality in critically ill patients

1, We confirmed that decreased Ca and increased osmolality can refer to increased mortality risk in septic patients.

2, We demonstrated that in nonseptic patients higher whole blood viscosity, increased red blood cell aggregation and the decreasing tendency of red blood cell deformability could be associated with poor outcome.

3, We suggested that these parameters may be added to Apache and SAPS scores to have a more accurate prediction.

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