Hemorheological investigations in carotid artery stenosis and in critically ill patients

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Prologue

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.

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.

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. Previous findings showed that these alterations can refer to the prognosis of the patients.

Methods

Hematocrit (Hct) was measured in a Haemofuge microhematocrit centrifuge (Heraeus; Germany) using native capillaries. Measurements were performed at room temperature. Plasma fibrinogen concentration was determined by Clauss method. Whole blood viscosity (WBV) was determined with Brookfield DV-III Ultra LV cone-plate rotational viscometer (Brookfield Engineering Laboratories Inc, Middleboro, USA). Plasma viscosity (PV) was measured with a Hevimet 40 capillary viscometer (Hemorex Ltd., Budapest, Hungary). Plasma was collected after whole blood sample centrifugation for 10 minutes at 1500g. Measurements were performed at 37 °C. Red blood cell aggregation measurements were carried out with a LORCA aggregometer (Laser-assisted Optical Rotational Cell Analyzer; R&R Mechatronics, Hoorn, The Netherlands) and Aggregation Index (AI), aggregation half time (t ½) and threshold shear rate (γ) were calculated. We measured red blood cell deformability with LORCA ektacytometer.

Hemorheological alterations in carotid artery stenosis

Introduction

Carotid artery stenosis 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.

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

In chronic cerebrovascular disorders chronic hyperviscosity and increased fibrinogen level are present; furthermore, impaired deformability and elevated RBC aggregation were also found. Previous studies in the past 30 years suggest that altered hemorheology can correlate with the degree of carotid artery stenosis.

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.

Subjects and Methods

107 patients (44 males, 63 females, mean age 64±6 years) were recruited in the study. 40% of patients had ischemic stroke or transient ischemic attack in their history (symptomatic group). Based on carotid ultrasonography, patients were divided into non-stenotic group and stenotic group. Non-stenotic patients were further divided into three groups: (1) negative group, (2) evolving atherosclerosis (AS) group, and (3) minimal stenosis; and stenotic patients into two groups: (4) moderate CAS and severe CAS or occlusion.

Blood samples were taken from the antecubital vein. Routine laboratory and hemorheological parameters were determined. Hemorheological measurements were carried out within 2 hours after blood sampling, according to the above mentioned methodological description (see page 2).

Statistical analysis was performed with IBM SPSS statistical software version 22. Data are expressed as means ± SD. Relationship of the 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 $p < 0.05$.

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, but there were no differences in fibrinogen level or other routine laboratory tests.

Hematocrit was not different between non-stenotic and stenotic group, neither between asymptomatic and symptomatic group. Whole blood viscosity 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 or without stenosis (Figure 1).

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.

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, what refers to a higher red blood cell aggregation.

Worse red blood cell deformability 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.

Data of the CAS subgroups showed impaired red blood cell deformability and elevated PV 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.

In the stenotic group we found Hct, PV, and WBV higher in smokers.

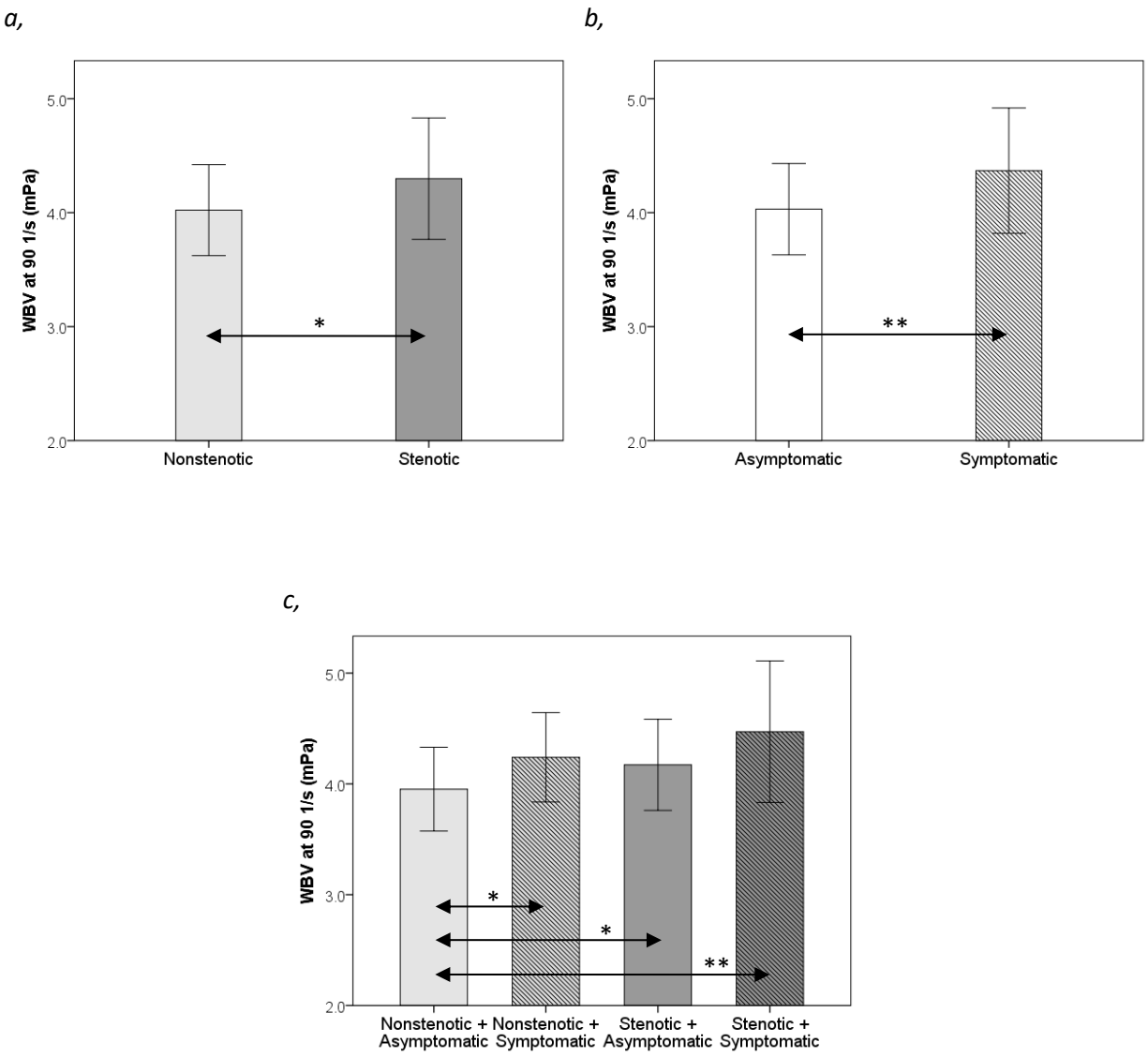


*Figure 1: Differences between whole blood viscosity levels (a, nonstenotic – stenotic; b, asymptomatic – symptomatic; c, subgroups compared to nonstenotic and asymptomatic patients). Values are means ± SD. *p<0.05, **p<0.01*

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.

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.

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.

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.

The relationship between hemorheological parameters and mortality in critically ill patients

Introduction

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. 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. Red blood cell aggregation is increased in sepsis and it correlates with prognostic scoring systems. Macrorheological factors, like whole blood viscosity and plasma viscosity can be altered in septic and also in nonseptic patients. Only one recent study researched these variables in correlation with outcome and highlighted the potential effect of microrheology.

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.

Subjects and methods

112 patients treated in intensive care unit (ICU) with different non-surgical diseases were recruited. 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, and the change of values (Δ) was calculated. At ICU admission the presence of sepsis 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) were calculated. Mortality was followed up to 30 days. ICU mortality was 37.5%, while 30-day mortality was 46.6%.

Arterial blood was collected and routine laboratory parameters were determined. Hemorheological measurements were carried out within 2 hours after blood sampling, according to the above mentioned methodological description (see page 2).

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 $p < 0.05$.

Results

Heart rate was higher, blood pressure was lower in nonsurvivors. Kaplan-Meier analysis and Cox proportional hazard model represented increased changes in Ca level with about twice higher mortality risk and patients with Δosmolality above the median have about 3-times lower survival rate.

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.

There was no difference in capillary hematocrit level between survivors and nonsurvivors. In nonseptic patients Kaplan-Meier analysis showed that patients with WBV values above the median had poorer survival, with about 4-times higher mortality risk according to Cox proportional hazard model (Figure 2a).

Among nonseptic patients, nonsurvivors showed increased RBC aggregation in the first day. Kaplan-Meier analysis showed a significant difference in 30-day survival of patients with aggregation above or below the median in the total population. It was more prominent in

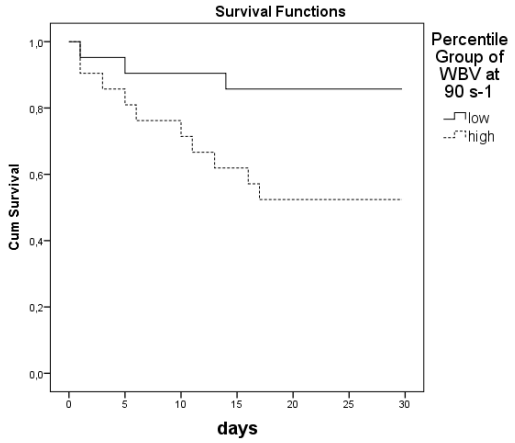
the nonseptic group with about 4-times higher Hazard ratio for higher aggregation, although in the septic group no difference was found (Figure 2c).

In nonsurvivors RBC deformability showed worsening from the 1st to the 2nd day, and it was found to be lower on the second day compared to survivors 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, no relationship was found in sepsis during subgroup analysis (Figure 2b). In nonseptic patients survival analysis represented 7-times higher mortality risk in patients with worsened RBC deformability.

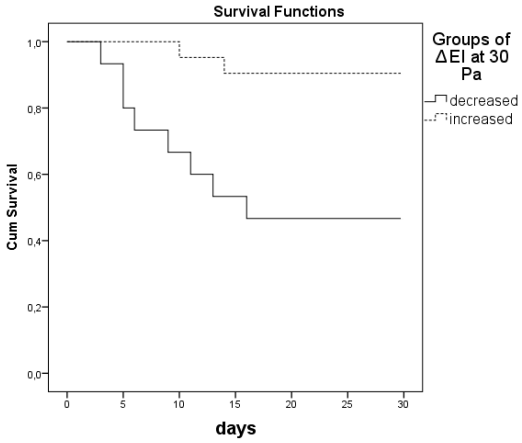
1st day fibrinogen was higher in sepsis and it decreased more than in nonseptic patients. Although the 1st day WBV and RBC aggregation were not different between septic and nonseptic patients, changes of WBV between the 1st and 2nd day decreased in sepsis, and changes of RBC aggregation increased in nonseptic patients. RBC deformability was impaired in septic patients both on the 1st and on the 2nd day.

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.

a, *WBV in nonseptic patients*



b, *ΔEI in nonseptic patients*



c, *AI in nonseptic patients*

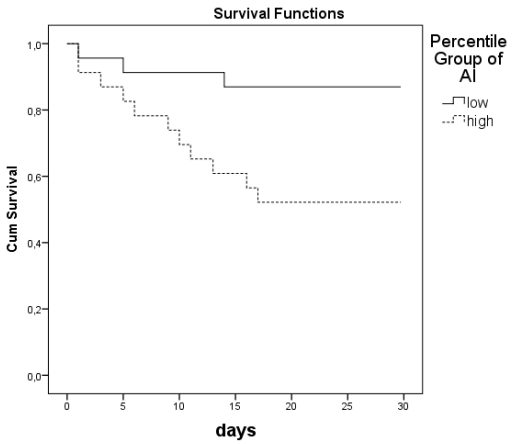


Figure 2: Survival (Kaplan-Meier) curves of nonseptic patients (a, whole blood viscosity; b, red blood cell deformability; c, red blood cell aggregation).

Discussion

Several researches investigated hemorheological parameters in critically ill patients in the past few decades. However, only a few studies can be found in correlation with survival or ICU scoring systems.

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 INR in nonsurvivors and that the change of Ca and osmolality could refer to survival, because these parameters are not in any scores.

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. 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. 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.

Several previous researches have explored impaired red blood cell deformability in sepsis and also revealed it as a marker of severity, it was lower than in nonseptic patients at higher shear stresses as our study indicated. 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.

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.

Summary of the new scientific results

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.

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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The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pecs, Hungary.

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Impact factor (2015): 2.242

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