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Red blood cell deformability in patients with human immunodeficiency virus infection

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Abstract Red blood cell (RBC) deformability is a major determinant of the ability of the RBC to pass repeatedly through the microcirculation. A decrease in RBC deformability leads to tissue perfusion and organ dysfunction. The purpose of this study was to measure the rigidity of RBCs from human immunodeficiency virus (HIV) seropositive individuals and investigate its relation to immune status and viral load. A filtration method based on the initial flow rate principle was used to determine the index of rigidity (IR) of 53 samples from HIV patients and 53 healthy individuals. The mean IR was significantly increased in patients with HIV compared to healthy individuals ($P < 0.01$). IR was inversely correlated with current CD4⁺ T-lymphocyte counts ($P < 0.0001$). High CD4 cell counts (>200 cells/ μ l) are related to low IR values, independently of the viral load (VL). No differences in rigidity were noted between the VL groups, although there was a trend towards an increased IR in patients with high VL within the group of CD4 <200 . RBC deformability is decreased in HIV disease, in a degree mainly related to CD4 depletion. Further studies are needed to elucidate the underlying mechanisms and the role of VL in highly immunocompromised HIV patients.

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Introduction

Human immunodeficiency virus (HIV) infection is characterized by a brisk immune activation originated by the increase of virus amount to high levels in its first flu-like stage. Afterwards, in a second asymptomatic period for the patients, the initial high level viremia disappears and the disease is characterized by CD4 cell depletion and immune dysfunction in patients with advanced disease and acquired immunodeficiency syndrome (AIDS). During the asymptomatic period of the disease, in which little appears to be happening macroscopically, many changes in the blood component properties (e.g. deformability, aggregability, viscoelasticity) take place [1]. Particularly, changes may occur in hemorheological parameters, such as erythrocyte and leukocyte rigidity as well as erythrocyte aggregation, hematocrit and plasma viscosity. In many studies the virus load measurements by themselves do not provide information about the asymptomatic period as it is a quasi steady stage, but with high viral turnover rates for both free virus and productively infected cells and profound underlying dynamic processes [1, 2].

One of the challenges in HIV research has been to unravel the multifactorial structure of the disease, which leads to changes in hemorheological parameters, such as the above mentioned.

In the literature the blood cell abnormalities and changes in their rheological properties have been reported in different diseases but are poorly investigated in the HIV infected population [3].

A wide variety of cardiac complications, which may result from either opportunistic infections, tumors or a specific involvement by the virus itself, has been recognized in HIV infected individuals. The effects of hemor-

heologic abnormalities are most significant in the microcirculation due to the similarity of red blood cell diameter and aggregate size to the vessel diameter [4, 5]. Microcirculation alterations with significant damage to the endothelial cells were observed in asymptomatic HIV patients [6].

Anatomic abnormalities of the microvasculature in HIV disease as well as different homological factors influence the dynamics of blood flow. The hematological factors which are referred to as the main hemorheological parameters associated with the disease are increased erythrocyte aggregation, increased leukocyte rigidity and decreased erythrocyte deformability [7, 8]. Increased erythrocyte aggregation and leukocyte rigidity are directly associated with increased plasma fibrinogen, which is a determinant factor in the HIV infected individuals. Fibrinogen levels have been associated with disease progression and CD4 depletion (degree of immunosuppression). Decreased erythrocyte membrane deformability associated with HIV infection may further affect tissue perfusion leading to organ dysfunction [8].

Recent studies found red blood cell (RBC) deformability significantly decreased in HIV infected individuals [7]. These studies suggested that increased aggregation and decreased deformability of RBC are features of HIV disease but unrelated to the severity of the immunodeficiency.

RBC aggregation is particularly apparent under conditions of low flow, namely, low shear stress [9], where rouleaux formation can dramatically decrease blood flow velocity leading to compromised tissue perfusion and oxygen delivery leading eventually to organ ischemia. Increased RBC aggregation has been associated with the severity of HIV disease [7, 8].

According to published findings, we hypothesized that the decreased deformability of the RBCs is a feature of HIV disease and probably related to the severity of immunodeficiency. Hemorheological techniques offer tremendous potential for the investigation of rheological changes in blood in different diseases and for the studies of basic physiological process in blood cells. We addressed the above hypothesis, using a filtration method based on the initial flow rate principle, and we measured the rigidity of the red blood cell membrane in different severity groups of individuals infected with HIV. The decreased RBC membrane deformability, which is known to adversely affect tissue perfusion, as well as its association with HIV infection were studied. Furthermore, we investigated the relation of RBC deformability to the degree of immunosuppression and the viral load. The understanding of these hemorheological changes in HIV infected individuals may provide new insights in the pathogenesis of the disease related to the microcirculation alterations.

Patients and methods

Study population

Fifty-three HIV infected patients from the AIDS Unit of Patras University Hospital were consented for blood draw and use of medical file data after full explanation of the purpose of the study. All patients were newly diagnosed and none of them received any kind of antiretroviral treatment. Their mean CD4 (+) T-cells and viral load are shown in Table 1. The same number of healthy HIV negative volunteers matched for age and sex were used as controls. Patients and controls that were using medications known to alter hemorheological parameters were excluded from the study.

RBC preparation

All arterial or venous blood samples (3 ml) were anti-coagulated with EDTA (1 mg/ml). The blood after volume adjustment with phosphate-buffered saline solution was centrifuged at $200\times g$ for 10 min. Then the supernatant (plasma, white blood cells and platelets) was carefully collected and discarded. From the middle of the erythrocyte-rich sediment, a sample of 0.32 ml was aspirated and diluted in 6 ml phosphate-buffered saline to obtain a final RBC suspension with hematocrit (Hct) of 5%. All measurements were completed within 2 hours after venipuncture.

Filterability method

We used a filtration method for evaluating possible rheological changes in the mechanical properties of RBCs from patients with HIV. In particular the filtration time, t , measurements were performed using the initial flow rate of RBC suspension and the suspending medium flow rate, induced by gravity forces, as previously described [10]. The apparatus, the hemorheometer, mainly consists of a central capillary, which is surrounded by circulating water with a constant temperature of 37°C . The lower section block holds the nuclepore membrane (diameter 13 mm; pore diameter $5\ \mu\text{m}$). The central capillary can be filled by either a RBC suspension or the suspending medium (buffer PBS-30). An electronic chronometer, which is actuated and stopped by two level detectors, measures the time lapse of suspension, t_s , or the time lapse of the buffer, t_b . The difference of the time lapses ($t_s - t_b$) is a measure of the difficulty of the RBC suspension to pass through the same nuclepore membrane and it determines the index of rigidity (IR) by the following relationship:

$$\text{IR} = \frac{(t_s - t_b)}{t_b} \cdot \frac{1}{H_{ct}}$$

where H_{ct} is the haematocrit of the suspension, expressed as a percentage. The above equation holds in the haematocrit

Table 1 Laboratory values of HIV patients and controls

Variable	All HIV-infected subjects	HIV-negative control subjects	<i>P</i> ^a
Number of subjects	53	53	
Index of rigidity	13.09±5.22	8.76±1.85	<0.001
CD4+ T-lymphocyte counts (cells/μl)	279.64±125.22	NE	
Viral load (copies/ml)	71230.90±22204.99	NA	

Data are expressed as the mean ± SD

NE not examined, NA not applicable

^a Student's *t*-test comparisons between HIV-infected and control subjects

range of 5%, so $H_{ct}=0.05$. High IR values indicate low RBC deformability. IR is a measure of RBC membrane deformability changes, which imply reorganization of the membrane structure. The relation between filterability and deformability of RBCs has already been discussed [11].

Filterability measurements

Filterability studies were blindly performed with gentle shaking of the RBC suspensions to prevent erythrocyte sedimentation during the filtration measurements. The nucleopore membrane was calibrated by measuring the filtration time, t_b , of the buffer; then, without changing or moving the filter, the filtration time of the RBC suspension, t_s , is determined. Thus, for each blood sample the index of rigidity (IR) was calculated by the above equation.

Statistical analysis

Statistical analyses were performed using statistical software SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Results were considered as significant at $P<0.05$. All comparisons between HIV-infected subjects and control subjects were conducted using Student's *t*-test. Differences among subgroups of HIV-infected subjects were assessed using analysis of variance (ANOVA), and individual paired comparisons were analyzed post hoc with Sheffe's test.

Results

Table 1 summarizes laboratory values for all subjects. RBC deformability was significantly reduced in HIV-infected individuals compared to HIV-negative control subjects ($P<0.001$).

RBC rigidity in HIV-infected individuals was inversely correlated with current CD4+ T-lymphocyte counts ($r=-0.835$, $P<0.0001$), indicating that RBC rigidity was greater for individuals with lower CD4+ T-lymphocyte counts (Fig. 1).

We also compared RBC rigidity between three subgroups of HIV-infected subjects, based on CD4 count (Table 2), using ANOVA. Significant differences were found when the group of $CD4<200$ cells/μl compared to the other two groups ($P<0.001$). However, when comparing IR between the group of $200<CD4<400$ cells/μl and the $CD>400$ cells/μl group, no statistically significant difference was found ($P=0.61$).

Finally, we compared IR in the three study subgroups in relation to the viral load ($VL<50$, $50<VL<10000$, $VL>10000$ copies/ml). There were no significant differences between RBC rigidity values in the three groups, although there was a trend towards an increased IR in patients with $CD4<200$ cells/μl and high (>10000 copies/ml) VL (Table 3).

Discussion

Altered blood cells rheological behavior has been proven to contribute to microvascular disease. Studying the changes of the rheological properties of blood cells, mainly of RBC and leukocytes, may provide an insight into the pathogenesis of the microvascular abnormalities associated with the HIV infection [12, 13]. RBC deformability and especially

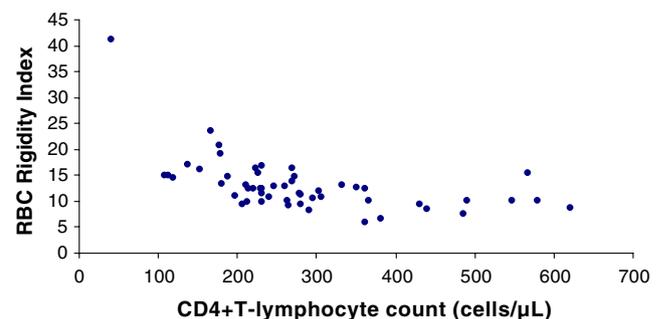


Fig. 1 Red blood cell rigidity index versus CD4+ T-lymphocyte count in HIV infected individuals. There is a statistically significant correlation ($r=-0.835$, $P<0.0001$)

Table 2 Comparison of RBC rigidity between patients grouped by level of CD4 count

Variable	CD4<200	200<CD4<400	CD4>400
Subjects (n)	12	33	8
CD4+ T-lymphocyte counts (cells/ μ l)	145.75 \pm 45.16	270.21 \pm 50.63	519.38 \pm 125.22
Index of rigidity (IR)	18.51 \pm 7.92 ^{a,b}	11.84 \pm 2.58 ^{a,c}	10.11 \pm 2.35 ^{b,c}

Data are expressed as the mean \pm SD

^a CD4<200 vs 200<CD4<400; P <0.001

^b CD4<200 vs CD4>400; P <0.001

^c 200<CD4<400 vs CD4>400; P =0.61

RBC membrane (RBCM) deformability and RBCM viscosity are the properties which mainly characterize the blood flow, particularly in the microcirculation system. The above properties in combination with plasma viscosity, the mean level of fibrinogen in the plasma, which is responsible for erythrocyte aggregation, as well as RBCs aggregation, are factors which directly influence blood flow in vivo. These factors were found to be altered in HIV-infected individuals and could be used as markers of alteration of RBCM architecture and structure during the evolution of the disease [14–16].

The main purpose of this work was to investigate any possible dependence of RBCM rigidity on both immune status and viral load. A relation of the viral load to the RBC rheologic alterations has been previously reported. However, the mechanisms of this effect have been far from elucidated. Previous data link the enhanced retention of RBCs in the spleen of HIV patients with instability of cytoskeleton structure as well as low hemoglobin levels, which affect the shape of RBCs, with the macrophage stimulation and the release of cytokines [17].

Activated polymorphonuclear leukocytes (PMNs) have been reported to induce structural and functional changes in neighboring RBCs [18–20]. Activation causes increased PMN rigidity and secretory activity with the production and release of chemotactic agents, oxygen free radicals and proteolytic enzymes. As a result there is increased lipid peroxidation of RBCM and alteration in RBC membrane cytoskeleton with increased cross-linking between spectrin and hemoglobin making the RBCs less deformable. Persistent activation of PMN, reported in HIV infection,

may thus affect the rheological properties of RBCM resulting in the increase of their rigidity [15, 21, 22].

The most significant findings of this study, concerning the changes of the RBC rheological properties in HIV patients, in relation to both the CD4 lymphocyte count and the viral load, are the following:

1. RBCM deformability was significantly lower in HIV infected individuals as compared to controls.
2. The CD4 lymphocyte count is a major determinant of RBC deformability, as high CD4 cell counts (>200) are related to a decreased IR, independently of the viral load.
3. In the HIV patients subgroup with the most compromised immune function (CD4<200), the increased viral load further decreases RBC deformability and reversibly affects IR, although the above did not reach statistical significance, probably due to the small number of our patients.
4. In HIV patients with CD4>200 we found a decrease of IR, nearly as low as healthy controls, even in the case of a high viral load. That probably means that in such patients the immune function, as defined by the CD4 count, but not viremia, is the major determinant of RBC rheology.

In conclusion, our findings showed that RBC membrane deformability is decreased in HIV disease, in a degree mainly related to CD4 depletion. Further studies are needed to gain insight into the underlying mechanisms as well as the role of the viral load on RBC rigidity in highly immunocompromised HIV patients.

Table 3 Index of rigidity (IR) values of HIV patients within different groups of level of immune function according to the values of viral load. Differences did not reach statistical significance

CD4 count	Index of Rigidity (IR)		
	VL<50	50<VL<10000	VL>10000
CD4<200	15.66 \pm 2.96(n =7)	18.81 \pm 5.13(n =3)	28.02 \pm 18.64(n =2)
200<CD4<400	12.33 \pm 2.21(n =21)	11.79 \pm 2.72(n =7)	9.86 \pm 3.42(n =5)
CD4>400	10.72 \pm 2.41(n =6)	(n =0)	8.31 \pm 0.88(n =2)

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