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Increased erythrocyte aggregation and oxidative stress in patients with idiopathic interstitial pneumonia

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Abstract. *Background:* Hemorheological properties are important determinants of tissue oxygenation. Although hemorheological alterations in various lung diseases have been well-defined, no information is available about the effects of idiopathic interstitial pneumonia (IIP) on hemorheological parameters. *Objectives:* The aim of this study was to investigate hemorheological parameters (erythrocyte deformability, aggregation, and plasma viscosity -PV) and associated oxidative stress indices in patients with IIP. *Methods:* The study enrolled 31 patients (9 Idiopathic pulmonary fibrosis (IPF), 10 non-specific Interstitial Pneumonia (NSIP), 12 Cryptogenic Organising Pneumonia (COP) and 33 healthy controls. Erythrocyte deformability and aggregation were measured by an ektacytometer. PV was determined by a cone-plate rotational viscometer and oxidative stress via a commercial kit. *Results:* Erythrocyte aggregation, total oxidant status (TOS) and oxidative stress index (OSI) of IIP patients were higher than controls whereas erythrocyte deformability, PV and total antioxidant status (TAS) were unaltered. *Conclusions:* Increment of oxidative stress in IIP seems to depend on enhancement of oxidants, rather than alteration of antioxidants. The issue that, elevated erythrocyte aggregation may further impair tissue oxygenation by disturbing microcirculation in IIP, may be considered in the follow up and development of new treatment protocols for this disease. *(Sarcoidosis Vasc Diffuse Lung Dis 2016; 33: 308-316)*

Key words: idiopathic interstitial pneumonia; erythrocyte deformability; erythrocyte aggregation; viscosity, oxidative stress.

Glossary of abbreviations

AI: Aggregation Index
AIP: Acute Interstitial Pneumonia
CCP: Chronic Cor Pulmonale
COP: Cryptogenic Organising Pneumonia
COPD: Chronic Obstructive Pulmonary Disease
CRP: C-Reaktif Protein
DIP: Desquamative Interstitial Pneumonia
DL_{CO}: Carbon Monoxide Diffusion Capacity Of The Lung
EI: Elongation Index
FEV_1: Forced Expiratory Volume In The First Second
FVC: Forced Vital Capacity,
Hb: Hemoglobin
Hct: Hematocrit
IG A: Immunoglobulin A
IG G: Immunoglobulin G
IG M: Immunoglobulin M

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IIP: Idiopathic Interstitial Pneumonia
IPF: Idiopathic Pulmonary Fibrosis
LIP: Lymphocytic Interstitial Pneumonia
MCH: Mean Corpuscular Hemoglobin
MCHC: Mean Corpuscular Hemoglobin Concentration
MCV: Mean Corpuscular Volume
NSIP: Non-Specific Interstitial Pneumonia
OSI: Oxidative Stress Index
Pa: Pascal
PaO_2: Partial Pressure Of Oxygen
Plt: Platelet Count
PV: Plasma Viscosity
RBC: Red Blood Cell
RB-ILD: Respiratory Bronchiolitis-Related Interstitial Lung Disease
ROS: Reactive Oxygen Species
SE: Standard Errors
SOD: Superoxide Dismutase
T 1/2:Aggregation Half Time
TAS: Total Antioxidant Status
TOS: Total Oxidant Status
WBC: White Blood Cell Count,
WBV: Whole Blood Viscosity

1. Introduction

Idiopathic interstitial pneumonia (IIP) includes a number of diseases which have different definitions with many common and also many different characteristics. Idiopathic pulmonary fibrosis (IPF, histologically termed Usual Interstitial Pneumonia (UIP), non-specific Interstitial Pneumonia (NSIP), Desquamative Interstitial Pneumonia (DIP), Respiratory Bronchiolitis-Related Interstitial Lung Disease (RB-ILD), Cryptogenic Organising Pneumonia (COP), Acute Interstitial Pneumonia (AIP) and Lymphocytic Interstitial Pneumonia (LIP)] are some well- known types of types of IIP (1, 2). IIPs other than IPF are related with inflammatory events and anti-inflammatory medication is beneficial in these cases (3). An important role of oxidative stress in the pathogenesis of IIP has also been demonstrated (4-8).

Lung tissue is exposed to a variety of stresses. Because of their large surface area and rich vascular bed, lungs are highly susceptible to reactive oxygen species (ROS) (7-9). On the contrary, lungs also have a large reserve of antioxidant agents such as glutathione and superoxide dismutase to counter oxidants (10). There is increasing evidence that oxidant-antioxidant imbalance is also associated with a variety of lung diseases such as chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome, lung fibrosis, lung infections and even malignant lung diseases (4, 9, 11).

Hemorheology is a branch of biorheology that deals with blood flow properties as well as the relationship between the vessel and the flowing blood (12). The flow properties of blood play significant roles in tissue perfusion by contributing to hydrodynamic resistance in blood vessels. These properties are influenced by pathophysiological processes, thereby increasing the clinical relevance of blood rheology information (13). Main components of hemorheology are erythrocyte deformability, red blood cell (RBC) aggregation, hematocrit (Hct), whole blood viscosity (WBV) and plasma viscosity (PV) (12, 14). Hemorheological parameters are known to be altered in a variety pulmonary diseases (15-17). In COPD patients, which is the most common lung disease, RBC alter morphologically, like cytoskeleton changes, ultra-structural modifications and reductions of glycophorin A (18-20). Hemorheological parameters of these patients were also shown to be modified depending on ROS overproduction, which is an important indicator in the pathogenesis of COPD (21-23). Being among the most susceptible cells to oxidative stress throughout the circulation, RBC were proposed as biosensors for the stadium of COPD (20, 24, 25). Increased oxidative stress in cor pulmonale and COPD results in decrement of erythrocyte deformability, leading to shortage of life span and thus hypoxia (17, 26, 27). On the other hand, rheological blood properties were also demonstrated to progressively deteriorate as pulmonary insufficiency increases in patients with chronic bronchitis and silicosis (18,19).

Hemorheological parameters have never been studied in IIP. Based on the above discussed data, we hypothesized that hemorheological parameters may also be modified in IIP, which is also a lung disease closely associated with oxidative stress. The study of hemorheological alterations and related oxidative stress in IIP may provide new insights into etiopathogenesis of the disease and favour discovery of new life-saving treatments.

2. Methods

2.1. Subjects

All the patients with a definitive diagnosis of IIP which were followed in the Chest Disease Department were enrolled in the study. Charts of 75 patients were evaluated in a retrospective manner and 31 IIP patients responded the call. Results were compared with 33 healthy volunteers with comparable age and gender distribution. The study enrolled 9 IPF (3 female, 6 male and mean age was 64.444 ± 3.153 years) patients, 10 NSIP (5 female, 5 male and mean age was 61.100 ± 3.060 years) and 12 COP (11 female, 1 male and mean age was 55.833 ± 2.587 years) patients and 33 healthy controls. Patients who participated to the study were all in stable period. Patients with exacerbations and under treatment were excluded. Study was approved by the Ethical Committee with the registry number 60116787-020/28435. All participants gave their written informed consent to the study.

2.2. Samples and Measurements

Venous blood samples of 10 mL were drawn by venipuncture into standart tubes containing EDTA (1000/cs, 100/cp) after 8 hours of fasting. Samples were appropriately transported to the Physiology Laboratory and hemorheological tests were performed within 3 hours in accordance with "new guidelines for hemorheological laboratory techniques" (28). Hematological parameters as complete blood count were determined by an electronic hematology analyzer (Siemens ADVIA® 2120i System, Siemens Healthcare Diagnostics, Japan). Serum C-Reaktif protein (CRP) and immunoglobulin (Ig) levels were mesured with by colourimetric method [Roche Cobas 8000, Roche Hitachi Moduler (Germany)]. Sodium citrated blood samples were analyzed for plasma fibrinogen concentration using a fully automated coagulometer [Beckman COULTER Acl top 700, instrumentation laboratory (USA)].

2.2.1. Erythrocyte Deformability Measurements

RBC deformability was determined at various fluid shear stresses by laser diffraction analysis using an ektacytometer (LORCA; RR Mechatronics, Hoorn, The Netherlands). The system has been described elsewhere in detail (29). Briefly, a low Hct suspension of RBC in an isotonic viscous medium (4% polyvinylpyrrolidone 360 solution; MW 360 kD; Sigma P 5288; St. Louis, MI) was sheared in a Couette system composed of a glass cup and a precisely fitting bob, with a gap of 0.3 mm between the cylinders. A laser beam was directed through the sheared sample, and the diffraction pattern produced by the deformed cells was analyzed by a microcomputer. On the basis of the geometry of the elliptical diffraction pattern, an elongation index (EI) was calculated as EI = $(L - W)/(L + W)$, where L and W are the length and width of the diffraction pattern, respectively. EI values were determined for 9 shear stresses between 0.3 and 30 Pa and similar patterns of RBC deformability alterations were obtained between groups at all stress levels. All measurements were carried out at 37°C.

2.2.2. Erythrocyte Aggregation Measurements

RBC aggregation was also determined by LORCA as described elsewhere (29). The measurement is based on the detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a computer-assisted system at 37°C. Backscattering data were evaluated by the computer and the aggregation index (AI) and the aggregation half time (t1/2) were calculated on the basis that there is less light backscattered from aggregating red cells. Aggregation measurements were determined using RBCs in autologous plasma adjusted to 40% Hct and blood was fully oxygenated before the measurements.

2.2.3. Determination of Plasma Viscosities

PV was determined with a Wells-Brookfield cone-plate rotational viscometer (model DV-II+Pro; Brookfield Engineering Labs, Middleboro, MA) at shear rates of 375 s^{-1} at 37°C.

2.2.4. Determination of total oxidant status (TOS)

The serum total oxidant status (TOS) was measured using a novel automated colorimetric measurement method for TOS developed by Erel (30). In this method, oxidants present in the sample oxidize

the ferrous ion O-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules (e.g., lipids, proteins) present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L/mg protein).

2.2.5. Measurement of total antioxidant status (TAS)

The total antioxidant status (TAS) of the serum was measured using a novel automated colorimetric measurement method for TAS developed by Erel (31). In this method the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the tissues, preventing the color change and thereby providing an effective measure of the tissue TAS. The results are expressed as the mmol Trolox/mg protein.

2.2.6. Calculation of oxidative stress index

The ratio of TOS to TAS is referred as oxidative stress index (OSI). The OSI is calculated according to the following Formula;

OSI (arbitrary unit) = TOS (μmolH_2O_2 Equiv. /L) / TAS (mmol Trolox Equiv./L) X 100 (32).

2.3. Statistical Analyses

All statistical analyses were performed using SPSS 21.0 software. Shapiro–Wilk's test was used for determination of normal distribution. Continuous variables were defined by the mean ± standard errors (SE). Mann–Whitney U test and Independent Samples t test was used for comparison of continuous data. Intergroup comparisons of categorical parameters were made using chi-square test. Spearman›s correlation coefficient was used for to examine the correlation between continuous variables. *p* values <0.05 were accepted as statistically significant.

3. Results

Table 1 shows the demographic characteristics and laboratory data of the groups. The study enrolled 31 IIP patients (19 female, 12 male) (mean age was 60.032 ± 1.747 years) and 33 age and sex matched healthy controls (18 female, 15 male) (mean age was

Table 1. Demographic characteristics and laboratory data of the groups

Parameter	Idiopathic interstitial pneumonia (n=31)	Control group (n=33)	p
Age (years)	60.032±1.747	56.818±2.157	0.134
Gender (F/M)	19/12	18/15	0.585
Hb (g/dl)	13.458±0.285	13.997±0.353	0.244
Hct (%)	41.654±0.792	42.072±0.978	0.743
RBC (m/μL)	4.747±0.112	4.874±0.099	0.398
MCV (fL)	88.287±1.331	86.312±0.977	0.154
MCH (pg)	28.529±0.490	28.715±0.423	0.742
MCHC (g/dl)	32.303±0.224	33.245±0.216	**0.001***
WBC (K/uL)	9.104±0.644	7.600±0.326	0.214
Plt (K/uL)	246.806±15.701	251.636±12.161	0.464
Fibrinogen (mg/dL)	321.233±17.900	324.848±17.863	0.831
CRP (mg/L)	2.537±1.652	0.808±0.439	0.177
IG M (mg/dL)	154.145±26.185	96.200±8.475	0.086
IG G (mg/dL)	1351.580±90.510	1032.193±34.101	**0.003***
IG A (mg/dL)	347.000±36.752	253.468±21.725	**0.041***

Values are expressed as means±SE (Hb: hemoglobin, Hct: hematocrit, RBC: red blood cell count, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell count, Plt: platelet count, CRP: C-Reaktif protein, IG M: immunoglobulin M, IG G: immunoglobulin G, IG A: immunoglobulin A). *p<0.05: the difference from control

56.818 ± 2.157 years) (p=0.134). Mean corpuscular hemoglobin concentration (MCHC) of IIP patients was lower (p=0.001), whereas Ig G and Ig A values were higher (p=0.003, p=0.041, respectively) compared to control group. There was no other statistically significant alteration between the parameters demonstrated in Table 1 (p>0.05). Table 2 shows partial pressure of oxygen (PaO_2) and pulmonary function test results of patients with IIP.

Erythrocyte deformability (i.e., the elongation index, EI) for the RBCs of the experimental groups was measured at nine shear stresses between 0.3 and 30.0 pascal (Pa) and shown in Table 3. The differences between the groups regarding EI values were not statistically significant (p>0.05).

Erythrocyte aggregation parameters (AI, $t_{1/2}$) and PV are demonstrated in Table 4. AI values of patients were higher than controls (p=0.003) while $t_{1/2}$ values were lower (p=0.016). The increments observed in AI of aggregation is concordant with the decrement in $t_{1/2}$ and indicate increment of RBC aggregation. No statistically significant alteration was in terms of PV of the groups.

TOS and OSI of the patients were increased (p=0.020, p=0.022, respectively) compared to healthy controls, whereas TAS was unaltered (Table 5). The correlation analysis showed a negative correlation between TOS values and EI measured at 1.69 Pa (r=-0.398, p=0.029) in IIP group. A positive correlation between TAS and RBC deformability measured at 5.33 Pa was also determined in these patients (r=0.377, p=0.040). No significant associations between the measured parameters were obtained in control group (Correlation datas were not shown).

Table 2. PaO_2 and pulmonary function test results of patients with IIP

	Mean ± SE	Medyan (min- max)
FEV_1 (L) (n=31)	1.853 ± 0.112	1,86 (0,7 - 2,88)
FEV_1% predicted (n=30)	80.767 ± 3.798	77 (43 - 140)
FVC (L) (n=30)	2.297 ± 0.117	2,28 (0,8 - 3,63)
FVC % predicted (n=30)	78.667 ± 3.213	76,5 (47 - 137)
FEV_1 /FVC ratio (n=30)	85.100 ± 1.102	85 (75 - 98)
PaO_2 (mm/Hg) (n=26)	70.831 ± 2.613	70 (47 - 90)
DL_{CO} % (n=29)	71.379 ± 2.703	75 (29 - 91)

Values are expressed as means±SE. (FEV_1: Forced Expiratory Volume in the First Second, FVC: Forced Vital Capacity, PaO_2: Partial Pressure of Oxygen, DL_{CO}: Carbon monoxide diffusion capacity of the lung)

Table 3. The EI values of the groups at different shear stresses

Shear stress (Pa)	Idiopathic interstitial pneumonia (n=31)	Control group (n=33)	p
0.30	0.037±0.004	0.043±0.004	0.502
0.53	0.086±0.006	0.084±0.008	0.577
0.95	0.173±0.010	0.167±0.013	0.718
1.69	0.262±0.014	0.272±0.016	0.804
3.00	0.378±0.011	0.369±0.013	0.962
5.33	0.461±0.009	0.458±0.011	0.925
9.49	0.527±0.007	0.527±0.008	0.877
16.87	0.581±0.006	0.579±0.007	0.851
30.00	0.619±0.006	0.616±0.006	0.774

Values are expressed as means±SE; EI: elongation index, Pa: pascal

Table 4. The RBC aggregation and plasma viscosity (PV) measurements of the groups

	Idiopathic interstitial pneumonia (n=31)	Control group (n=33)	p
AI (%)	74.572±1.382	66.021±2.823	0.003*
t ½ (sn)	1.386±0.099	1.819±0.134	0.016*
PV (mPa.s) (375 s^{-1})	1.908±0.125	1.746±0.065	0.166

Values are expressed as means±SE; AI: aggregation index, t ½: aggregation half time, PV: plasma viscosity. *p<0.05: the difference from control

Table 5. TOS, TAS and OSI measurements of the groups

	Idiopathic interstitial pneumonia (n=31)	Control group (n=33)	p
TOS (μmolH_2O_2 Equiv. /L)	21.974±1.766	16.446±1.517	0.020*
TAS (mmol Trolox Equiv./L)	1.588±0.058	1.587±0.094	0.554
OSI (arbitrary unit)	1.538±0.144	1.133±0.131	0.022*

Values are expressed as means±SE; TOS: The total oxidant status; TAS: The total antioxidant status levels; OSI: The oxidative stress index levels. *p<0.05: the difference from control

4. Discussion

IIP is a heterogeneous group of lung disorders that evolve toward pulmonary fibrosis of variable severity (3, 33, 34). Due to the low incidence and difficulty in the diagnosis of IIP, there is limited data on the pathogenesis and epidemiological properties as well as the treatment options of the disease (35,36). The results of the current study demonstrate hemorheological alterations and their association with oxidative stress in IIP patients for the first time in the literature.

The delivery of oxygen to tissue depends on three factors: 1) the functional integrity of the pulmonary, cardiac and vascular system, 2) the quantity and quality of the hemoglobin molecule and binding of oxygen to hemoglobin and, 3) the flow characteristics of the blood (hemorheology) (37). Blood flow, RBC aggregation and deformability are main components of hemorheology. In the microcirculation, RBCs must collapse to pass through narrow capillaries; hence, RBC deformability and aggregation are major determinants of flow resistance. The ability of the entire RBC to distort is critically important to perform its function of oxygen delivery and is also a determinant of cell survival time in the circulation (12). RBC aggregation is a reversible process meaning a temporary linear or branched aggregate formation of the erythrocytes under critically low shear stress conditions. The physiological importance of erythrocyte aggregation which is the reversible adhesion of adjacent erythrocytes in circulation is its tendency to increase the blood viscosity in low shear flow and to disturb the passage in capillary circulation (38). AI of IIP patients were higher, whereas $t_{1/2}$ values were lower compared to healthy individuals in our study. When considered together, the alterations in these two parameters are concordant with each other and indicate increased RBC aggregation in patients with IIP. It is worthy to note here that, enhanced RBC aggregation in patients with IIP may further contribute impairment of tissue oxygenation and thus worsen the clinical condition of the patient.

Mechanical properties of RBC are dependent on the proper metabolic conditions and a normal homeostasis in their microenvironment (37,38). The fibrous proteins in the plasma, red cell membrane properties and the erythrocyte morphology are known as the most important determinants of erythrocyte aggregation (38-40). It was shown that erythrocyte aggregation occurs as a result of increasing amount of plasma proteins in the situations of tissue damage caused by inflammation, trauma, or changing nature of erythrocyte membrane caused by sepsis and tissue damage (38,41). Role of inflammation was proposed in the pathogenesis and progression of IIP (8,42). Increased IgG and IgA concentrations observed in the patients of the current study are in line with literature findings and may at least partly explain increased RBC aggregation in these patients.

A number of studies in the literature have shown increased oxidative stress in several lung diseases, including IIP (5-7, 43-45). On the other hand, during the development of pulmonary diseases, antioxidant responses are different. Some lung pathologies lead to an increase of antioxidant enzymes, whereas there are no changes found in the others (46,47). In contrast, superoxide dismutase (SOD) activity was shown to be significantly lower in patients with asthma and decreases further during exacerbation (48). Markart P et al. demonstrated that alveolar levels of nonenzymetic antioxidants are elevated in fibrosing lung diseases, but are incapable of restoring oxidative balance (49). In concordance with above mentioned studies, we found increased TOS and OSI levels in the patient group. Although TAS level was also slightly elevated in IIP patients, the difference was not statistically significant, demonstrating that the enhancement of oxidative stress in the case of IIP is mainly due to the increments of oxidants. Unlike previous studies, the measurement of TOS and TAS in our study practically represents the cumulative action of oxidant and antioxidants and their synergistic interaction, which reflects the total oxidant-antioxidant status as well as OSI of the organism (50).

Oxidative stress is also accepted as one of the determinants of erythrocyte aggregation (40,51). RBC are highly susceptible to oxidative damage due to the high cellular concentration of transition metal ions, molecular oxygen and oxyhemoglobin, a potentially powerful promoter for the oxidative processes (52). Erythrocytes are also exposed to free radicals from the extracellular environment such as activated leukocytes. Although leukocyte activation was not determined in the current study, white blood cell count (WBC) number was slightly increased in the patient group. Between 1 and 3% of oxygen consumed by aerobic cells is estimated to become ROS (53). RBC possess multiple enzymatic and non-enzymatic antioxidant defense mechanisms to prevent oxidative damage, but during oxidative stress, these mechanisms may become exhausted (54). Erythrocyte aggregation is known to increase by the effect of oxidative stress (40,51). It can be speculated here that, enhancement of RBC aggregation may -at least partly- be due to the elevation of oxidative stress in IIP patients. However, no correlation was demonstrated between erythrocyte aggregation and oxidative stress parameters.

Other hemorheological parameters determined in the current study are the erythrocyte deformability and PV. Decreased RBC deformability increases apparent blood viscosity and hence flow resistance in larger vessels (37, 41). PV plays an important role in the flow regulation and is the mean component of blood which is in direct contact with the vessle wall, due to the axial migration (37). RBC deformability was measured at nine shear stresses between 0.3 and 30.0 Pa, representing the shear stress erythrocyte encounters at different sites of circulation, and no statistically significant alteration was observed. Although a negative correlation between TOS and EI measured at 1.69 Pa and a positive correlation between TAS and RBC deformability measured at 5.33 Pa were determined in IIP patients, these correlations do not make much sense since they were statistically significant at just one shear stress for each parameter. Although PV of IIP patients were increased the alteration was not statistically significant as well. Increment of IgG and IgA in plasma of IIP patients may be a reason of this elevation. Up to our knowledge, there is no study in the literature investigating RBC deformability and PV in IIP.

Zhou et al. demonstrated increment of PV in silicosis (19). On the other hand, literature data about erythrocyte deformability in several lung diseases is controversial, for some authors have found it increased (55), while others found decreased (17, 56) or unchanged (26, 27). Increment of oxidative stress is known to lead decrement of RBC deformability (40). Reduced RBC deformability was demonstrated in diseases associated with increased oxidative stress such as COPD and cor pulmonale and hypoxia was linked to the impairment of RBC deformability (17, 26). The discrepant results may be due to the different developmental stage of the diseases and patophysiology as well as the measurement techniques. Many alterations in RBC of COPD patients were described, e.g. polyunsaturated fatty acids and cytoskeleton modifications and ultrastructural changes as well as reduced glycophorin A, band 3, RBC thiols and enhanced sphingomyelin values (24, 57, 58). Additionally, an increase of erythrocyte membrane fluidity was reported (57). Verbitskii ON et al studied 120 patients with chronic obstructive bronchitis at different developmental stage of cor pulmonale and they found neither PV nor RBC deformability were changed in compensatory chronic cor pulmonale (CCP). In CCP decompensation, the index of erythrocyte deformability was decreased while PV was increased (27). Unaltered PV and RBC deformability determined in our study may be related to our patients' being in the stable stage of IIP.

In conclusion, the results of the current study clearly demonstrate enhanced RBC agrregation and oxidative stress in patients with IIP. The rise observed in oxidative stress was mainly due to the increment of oxidants. In addition to the existing lung pathology, increased RBC aggregation in this disease may serve as an unfavorable factor to worsen the clinical condition of the patient by further disrupting tissue oxygenation even in the stable period of the disease. We propose that this issue should be kept in mind of the clinician during the follow up of IIP patients. This is a preliminary report with limited number of patients. Further research in various types of IIP may be necessary to confirm our results and clarify the mechanisms leading to increment of RBC aggregation and oxidative stress in IIP. A better understanding of the role of the intrinsic properties of blood in these patients might be relevant for potential future drug interventions to enhance the microcirculatory flow conditions improving the hemorheological profile.

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Authors' contributions:

Erhan Ugurlu, contributed to the study design and data collection, performed the statistical analysis, interpreted the data and drafted the manuscript. reviewed the radiology, assessed the severity of disease and assisted with manuscript composition

Emine Kilic-Toprak, contributed to the study design and data collection, performed the statistical analysis, interpreted the data and drafted the manuscript.

Goksel Altinisik, contributed to the study design, data acquisition and reading and approving the final manuscript.

Ozgen Kilic-Erkek, contributed to the the data collection and helped to draft the manuscript.

Betul Cengiz, contributed to the study design and data collection, performed the statistical analysis, interpreted the data and reading and approving the final manuscript
Vural Kucukatay, contributed to the data acquisition, statistical analysis, writing the manuscript and reading and approving the final manuscript.
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Ismail Hakki Akbudak, contributed to the performed the data collection and helped to draft the manuscript.
Yusuf Ekbic, contributed to the performed the data collection and helped to draft the manuscript.
Melek Bor-Kucukatay, contributed to study design and writing the manuscript and revision of manuscript for important intellectual content and reading and approving the final manuscript.
All authors read and approved the final manuscript

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