Interrelation of Blood Microrheological Parameters Measured by Optical Methods and Whole Blood Viscosity in Patients Suffering from Blood Disorders: a Pilot Study

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Abstract. The perfusion of tissues and blood flux strongly depend on several interdependent parameters, such as viscosity of blood, red blood cell aggregation and red blood cell deformation. In case of many blood disorders these parameters are altered. This pilot study aimed to investigate the relationship between whole blood viscosity and microrheological parameters in patients with essential thrombocythemia (ET), as well as to compare microrheological parameters in patients with ET, erythrocytosis and spherocytosis. Blood viscosity measurements were performed using a rotational viscometer, while microrheological parameters were assessed through laser aggregometry and laser diffractometry techniques. The findings revealed novel data of the correlation between whole blood viscosity and microrheological parameters of patients with ET. Overall, this study provides valuable preliminary evidence on the relationship between whole blood viscosity and microrheological parameters in the context of blood disorders.

Keywords: aggregation; red blood cell; deformation; viscosity; essential thrombocythemia; spherocytosis; erythrocytosis.

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1 Introduction

Blood plays a vital important role in supplying oxygen to body tissues and in transporting various substances throughout the body [1]. Therefore, the parameters characterizing blood flux reflect the quality of blood functionality. In general, there is a division between the interrelated macrorheological parameters, i.e., blood viscosity and blood flow velocity, and the microrheological parameters, i.e., red blood cell (RBC) aggregation and RBC deformability [2–4].

Blood consists of plasma and blood cells, i.e., RBCs, platelets, and leukocytes. Blood is a shear-thinning fluid, which means that its viscosity diminishes as the shear rate increases [3]. At low shear rates (<100 s^{-1}), blood viscosity is primarily affected by RBC aggregation, whereas at high shear rates (>100 s^{-1}) by hematocrit, RBC deformability, and plasma viscosity. RBC aggregation is a reversible process of formation of stack of coins-like linear and more complex structures called *rouleaux* [5]. RBC deformation is also reversible and plays a crucial role in the microcirculation since the average diameter of many terminal capillaries is smaller than the linear size of the RBC [4].

Deviation of the parameters related to the blood viscosity, i.e., RBC aggregation and deformation from the normal state, may be a result of pathological processes in the organism or disease complications [6–9]. Abnormal RBC aggregation and deformation may influence thrombotic events by promoting hemolysis, thrombophilia, inflammation, and microvascular occlusion [10].

In this work, we investigated groups of patients suffering from essential thrombocythemia (ET), erythrocytosis (ERY) and spherocytosis (SPH). ET and ERY belong to the group of chronic myeloproliferative disorders (CMD). Overall, CMD involve overproduction of different blood cell types in the bone marrow [11, 12]. Conditions like polycythaemia vera and primary myelofibrosis fall under this category. Essential thrombocythemia (ET) is characterized by overproduction of platelets in the bone marrow, leading to increased clotting risk and serious complications [13]. ERY refers to conditions with elevated red blood cell counts. Primary ERY is polycythaemia vera, where the bone marrow produces too many RBCs. Secondary ERY occurs as a response to low oxygen or other pathological alterations [14]. SPH is a hereditary condition characterized by the presence of spherical-shaped RBCs, which are more prone to destruction and can lead to anemia [15].

Haemostatic complications such as vascular thrombosis and haemorrhages significantly reduce the life duration of patients with CMD [16]. These patients commonly experience arterial or venous thrombosis, with arterial thrombosis being responsible for 60%−70% of events associated with myeloproliferative neoplasms [17, 18]. This type of thrombosis includes ischemic stroke, acute myocardial infarction, and peripheral arterial occlusion [18]. Numerous studies have investigated platelet aggregation, platelet function, bleeding [19–22], and cardiac complications associated with CMD [23]. However, research on the aggregation and deformation of RBCs in CMD patients has been comparatively limited, with less focus on this aspect compared to platelet aggregation. It was shown that the deformability of RBC is decreased, and the RBC aggregation is increased for patients suffering from polycythemia vera compared to the control group [24]. Also, the viscosity of whole blood is increased in polycythemia vera disease both for high and low shear rates [25]. Regarding the SPH, it was shown that deformability of RBC is decreased [26].

The goal of this work is to investigate for the first time the relationship between whole blood viscosity and microrheological parameters in patients with ET as well as to compare the microrheological parameters in patients with ET, ERY, and SPH.

2 Materials and Methods

2.1 Patients and Blood Samples Preparation

Overall, 18 patients with different blood disorders were enrolled in the study. The patients were divided into 3 groups based on their clinical diagnosis: ET group, ERY group, and SPH group. Parameters of the studied groups (mean age and number N) are presented in Table 1.

Blood samples were collected on an empty stomach from the patient's cubital vein into 2 ml tubes with EDTA K2 or EDTA K3 anticoagulants. All patients were informed of the purpose of the research and gave the informed consent in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Medical Research and Educational Center of M.V. Lomonosov Moscow State University (protocol No. 11/22 from 05.12.2022). The experiments were performed within 6 h after blood collection, at which time the aggregation and deformation properties of RBC remain relatively stable [27]. In this paper, we do not consider a control group of healthy donors.

2.2 Viscosimetry

The rotational viscometer Lamy Rheology RM100 CP1000 (Lamy Rheology Instruments, France) acquired in the frame of Moscow State University Development Program equipped with MK-CP40Z measuring systems was used to measure the viscosity of whole blood. All measurements were performed under the room temperature (T = 22 ℃). For all experiments, shear rate of 1000 s^{-1} was set to measure blood viscosity. The viscosity at this high shear stress is primarily a function of hematocrit, plasma viscosity, and RBC deformability [28]. Blood viscosity is strongly dependent on hematocrit, so we further normalized blood viscosity from different samples using the constitutive models of Krieger and Daugherty [29]. This model is often used to obtain effective viscosity of blood and estimate the optimal hematocrit [30, 31]. The Krieger and Dougherty model states the following Eq.:

Table 1 Parameters of the studied groups.

Group	Number	Number of males	Number of females	Mean age ± SD
ET	11	8	3	11.3 ± 4.3
ERY	3	2	1	8.3 ± 4.5
SPH	4	1	3	12.3 ± 3.9

$$\eta = \eta_0 \frac{1}{\left(1-\frac{\varphi}{\varphi_m}\right)^{2.5\varphi_m}}, \quad (1)$$

where φ_m is the max normalized volume of RBC in a suspension of plasma, φ is the current normalized volume of RBC in a suspension. For example, spheres can be packed with a maximum volume fraction of $\varphi_m \approx 0.74$. As far as RBC can deform the φ_m value is near the top limit ($\varphi_m \approx 1$). Thus, $\frac{\varphi}{\varphi_m}$ equals to the hematocrit. From Eq. (1), η_0 was calculated which corresponds to the theoretical viscosity of blood plasma. This calculated value is an additional parameter to normalize the whole blood viscosity by hematocrit and may not reflect the real plasma viscosity.

2.3 Laser Aggregometry

RBC aggregation was quantified using the diffuse light scattering technique implemented in the RheoScan-AnD300 device (RheoMediTech, Republic of Korea) [32, 33]. This method analyzes the scattered light obtained from a whole blood sample when illuminated by a laser beam ($\lambda = 635\,nm,\ P = 1.5\,mW$). Two types of disposable test chips were used to measure hydrodynamic strength of RBC aggregates and parameters of spontaneous RBC aggregation.

For performing the measurements of RBC hydrodynamic strength, a whole blood sample is placed into the reservoir connected to another reservoir by a thin microchannel ($\approx 200\,\mu m$) and the back-scattered light intensity is registered as the blood with continuously reduced velocity passes through (Fig. 1a). At high shear stress, RBC aggregates break up into smaller aggregates or single RBCs. When the shear stress decreases, the process of spontaneous aggregation of RBCs prevails. According to light scattering theory, the larger the particle size, the more light is scattered at small angles, and the forward scattering/backscattering ratio increases [34]. Thus, the changes of backscattered light intensity correspond to the changes in the mean size of RBC aggregates in the microchannel. The point of the maximum backscattered light intensity represents the balance between aggregation and disaggregation processes and is known as critical shear stress (CSS), which characterizes the hydrodynamic strength of RBC aggregates. The critical time (T_{crit}) refers to the specific moment when there is a balance achieved between RBC aggregation and RBC disaggregation (Fig. 1a).

The RBC aggregation kinetics is measured by registering the forward scattering intensity (Fig. 1b). In this case, the whole blood is placed in the microchamber, and the iron rod begins to rotate, resulting in complete disaggregation of the blood. When the rod stops, the kinetics of RBC spontaneous aggregation is measured and several parameters are calculated: the aggregation index (AI), characteristic time of RBC aggregation ($T_{1/2}$), AMP parameter, and M parameter [35, 36].

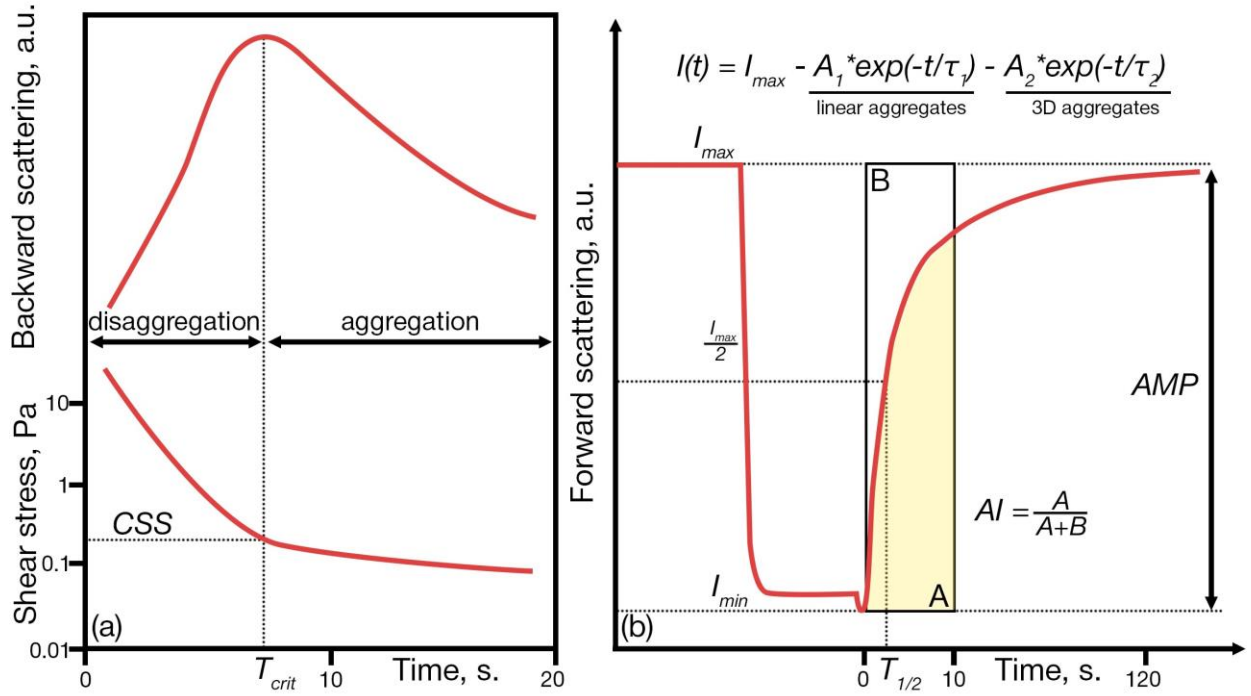

Fig. 1 (a) (below) The shear stress in the microchannel as a function of time; (above) the intensity of backward scattered light from the blood in the microchannel as a function of time. (b) The forward scattered light intensity ($I(t)$) from the whole blood sample in the chamber as a function of time (t) during RBC spontaneous aggregation. $I(t)$ is approximated by two exponential functions and linear aggregation (A_1,τ_1) and 3D aggregation (A_2,τ_2) parameters are calculated.

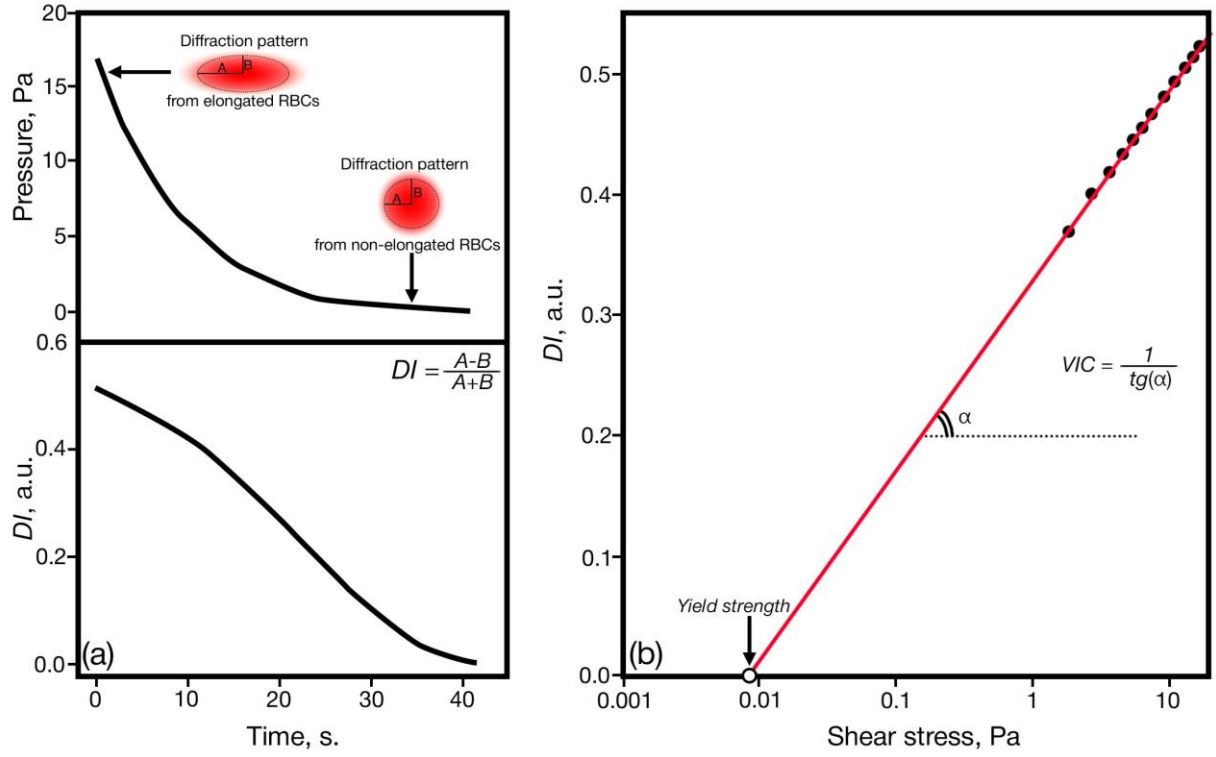

Fig. 2 (a) (below) The deformability index (*DI*) as a function of time (*DI* correspond to the relative elongation of RBCs); (above) the pressure difference at the ends of the microchannel in the microchamber as a function of time. (b) *DI* as a function of shear stress in semi logarithmic axes. The explanation of yield *Yield strength*, i.e., the minumim shear stress requred for RBCs elongation, and RBC intracellular viscosity (*VIC*) calculation. α is the angle of slope of the curve.

In detail, AI characterizes RBC spontaneous aggregation in the first 10 s as well as M parameter equals to the area A (Fig. 1b). AMP indicates the difference in scattered light between the non-aggregated RBC state and the fully aggregated RBC state. $T_{1/2}$ characterizes the time to reach half of the maximum intensity. The more the RBC aggregation, the higher the values of parameters AI, M, and AMP and the lower the value of the $T_{1/2}$ parameter.

The process of RBC aggregation is not a gradual process, i.e., linear and 3D aggregation occurs simultaneously. However, by utilizing approximation methods, it is possible to extract parameters that correspond to either linear or 3D aggregation. Additionally, the curve ($t > 0$) was fitted using biexponential curve (see Eq. in Fig. 1b). The τ_1 corresponds to the characteristic time of linear RBC aggregates formation, whilst the τ_2 corresponds to the characteristic time of 3-dimensional (3D) RBC aggregates formation.

2.4 Laser Diffractometry

RBC deformation was measured using laser ektacytometry technique which is also implemented in the RheoScan device (RheoMediTech, Republic of Korea) [37]. This method analyzes the diffraction patterns obtained from a diluted RBC suspension when illuminated by a laser beam (λ = 635 nm, P = 1.5 mW). As the RBCs elongate due to shear stress arising as a result of pressure difference at the ends of the microchannel, the diffraction pattern from the cells also elongates, allowing for the calculation of the deformation index (DI) (see Fig. 2a). This provides a quantitative measure of the cells' ability to deform under different shear stresses.

Additionally, the relationship between DI and shear stress in semi-log scale was approximated using linear regression, allowing for the calculation of *Yield strength* and viscosity of RBC intracellular content (VIC). The determination of these parameters was described by Firsov et al. [38]. The change in VIC can be estimated by the slope change in the DI vs. log of shear stress plot, while the *Yield strength* is determined by the intersection with the X-axis (see Fig. 2b). The *Yield strength* represents the minimum shear stress needed for RBC deformation, and the *VIC* corresponds to the viscosity of its content.

2.5 Statistical Analysis of the Data

Custom developed python applications were used to process and plot all the data. In the Results and Discussion Section, the box plots in the Figs. 3–5 show the first quartile (Q1) to the third quartile (Q3) of the data, with a median line inside. Each point in the figure corresponds to the average value for one patient of at least 3 measurements of AI, M AMP, and $T_{1/2}$; at least 7 measurements of η and CSS. The whiskers represent the standard deviations with the mean values (hole white points) at the center. To assess the statistical significance of differences between sample groups, the Mann-Whitney U test was conducted. Two sample groups were

considered significantly different if the *p*-value was less than 0.05. To determine the correlations between different parameters, Spearman's rank correlation coefficient was used. A correlation is considered weak if the absolute value of the coefficient was between 0.3 and 0.5, and strong if it was greater than 0.5.

3 Results and Discussion

3.1 Blood Viscosity, RBC Aggregation, and Deformation

The comparison of the measured parameters between ET, ERY, and SPH groups is presented in Figs. 3–5. No statistically significant differences in whole blood viscosity (shear rate = 1000 s^{-1}) were observed between the studied groups (Fig. 3). Nevertheless, the mean viscosity values at high shear rate for each group surpass the standard (normal) range of 3.5–5.5 mPa·s, as determined by many scientific groups [1]. Also, statistically significantly elevated hematocrit was observed in blood of patients with ERY (see Fig. 4). This is entirely consistent with medical evidence that ERY has a high hematocrit and/or hemoglobin level.

In Fig. 5 the dependence of *AI* parameter for 3 studied groups is presented. The statistically significant differences were found between ET and SPH groups ($p < 0.05$). It means that the RBC aggregation is reduced in patients with SPH and at the same time the blood viscosity is increased at high shear rates. This phenomenon can be explained by the fact that the shape of RBC in SPH is different from that of a biconcave disk and they deform much less. This in turn affects the RBC aggregation.

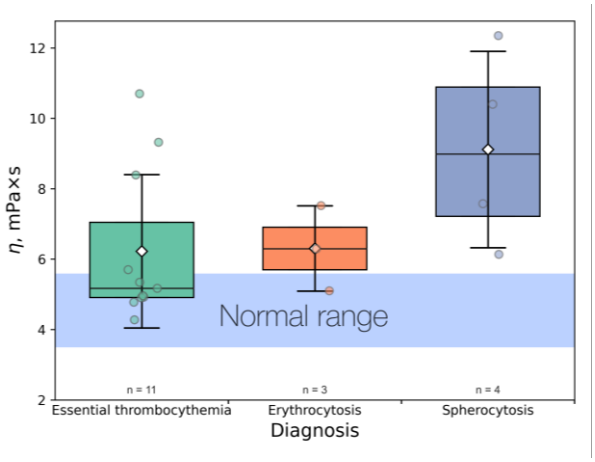

Fig. 3 Viscosity of whole blood (η) (shear rate = 1000 s^{-1}) for 3 studied groups of patients. Each point in the figure corresponds to the average value for a single patient. The bottom and top edges of the box correspond to the first (Q1) and to the third quartile (Q3) of the data with a median line inside. The whiskers are standard deviation. The rhombus point is the mean value. The normal viscosity range was determined from Ref. [1].

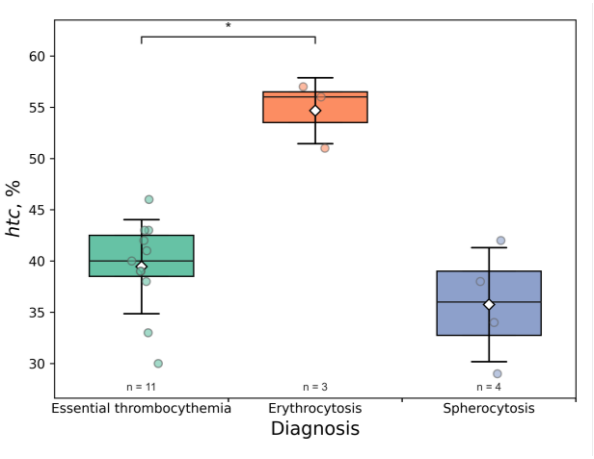

Fig. 4 Hematocrit (*htc*) for 3 studied groups of patients. Each point in the figure corresponds to the average value for a single patient. The bottom and top edges of the box correspond to the first (Q1) and to the third quartile (Q3) of the data with a median line inside. The whiskers are standard deviation. The rhombus point is the mean value. $^{*}p < 0.05$.

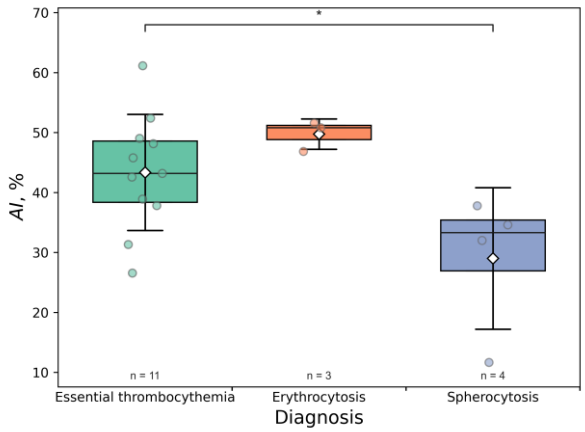

Fig. 5 *AI* parameter for 3 studied groups of patients. Each point in the figure corresponds to the average value for a single patient. The bottom and top edges of the box correspond to the first (Q1) and to the third quartile (Q3) of the data with a median line inside. The whiskers are standard deviation. The rhombus point is the mean value. $*p < 0.05$.

The other parameters characterizing RBC aggregation, i.e., M, AMP, $T_{1/2}$, τ_1, τ_2, CSS, were not statistically significantly different between the studied group. The further research is required to increase the number of participants and to add a control group of healthy volunteers in the study.

The deformability of RBCs statistically significantly decreased for the SPH group. This is most likelydue to the altered RBC shape in patients with SPH. The deformability of RBCs in the blood of patients with ERY is slightly lower than that of patients with ET but is not statistically significant. Further studies are required.

Table 2 Parameters of RBC deformability for the studied groups. Means ± SD are presented.

Group	*DI* (3 Pa), a. u.	*DI* (20 Pa), a. u.	*Yield strength*, Pa	*VIC*, a. u.
ET	0.30 ± 0.03	0.48 ± 0.02	0.19 ± 0.05	4.1 ± 0.1
ERY	0.28 ± 0.07	0.45 ± 0.07	0.28 ± 0.23	4.2 ± 0.2
SPH	0.25 ± 0.07	0.37 ± 0.08	0.16 ± 0.17	6.5 ± 2.1
Control from Refs.	0.32 ± 0.02 [39]	0.51 ± 0.01 [40]	–	–

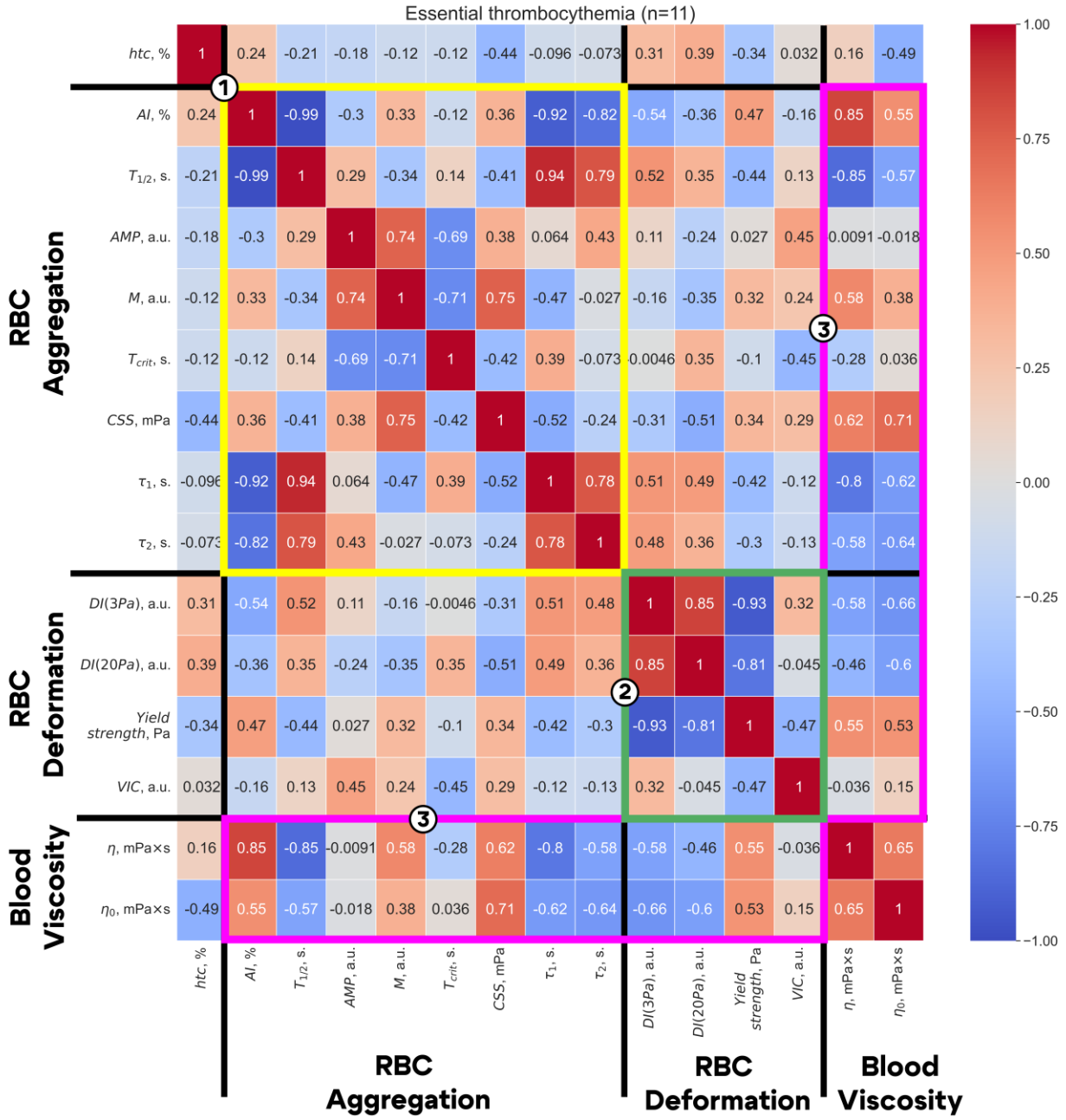

Fig. 6 Correlations between whole blood viscosity (η) (shear rate = 1000 s^{-1}), normalized viscosity (η_0), hematocrit (htc), and microrheological parameters for patients with ET. The correlations between parameters are characterized by the Spearman's rank correlation coefficient. The correlation matrix is divided by 3 groups of parameters, i.e., RBC aggregation, RBC deformation, and blood viscosity. Additionally, the correlations between RBC aggregation parameters are outlined by the yellow frame (#1); the correlations between RBC deformation parameters are outlined by the green frame (#2); the correlations between viscosity parameters and macrorheological parameters are outlined by magenta frames (#3).

Additionally, the mean values of the DI (3 Pa) and DI (20 Pa) parameters for all 3 groups are lower than in healthy adult subjects (see Refs. [39, 40]) (see Table 2).

Interestingly, the VIC parameter is elevated in patients with SPH. Although the work [26] demonstrated a lower spherocyte density, the increased VIC is likely due to the high intercellular heterogeneity of spherocytes.

The authors are not aware of any work that has investigated *Yield strength* and VIC using RheoScan. That is why the raw "Control from Refs." from Table 2 is absent for the two last columns. It is incorrect to compare *Yield strength* and VIC measured with other ektacytometers [38], since these parameters depend on the geometry of the measuring cuvette.

3.2 Correlation between Viscosity and Microrheological Parameters for Patients with ET

In this section, only patients with ET were considered because of the low number of patients with ERY or SPH (see Table 1). The blood microcirculation in terminal capillaries and the blood flow in large vessels play a critical role in the functionality of the circulatory system. The apparent viscosity of whole blood is a key property that determines blood fluidity. In certain diseases, such as the ones shown in Fig. 3, the apparent viscosity of blood is elevated. To understand the underlying reasons for this increased viscosity, it is important to analyze the correlation between viscosity and microrheological properties of the blood. These microrheological properties, including deformability and aggregation of RBCs, are responsible for the intrinsic properties of blood. By establishing such correlations, we can gain insights into the factors contributing to alterations in blood flow. Additionally, these findings may have clinical implications, as modifying the microrheological parameters that affect blood flow could potentially help correct the elevated blood viscosity.

In Fig. 6, the Spearman's rank correlation coefficients between the whole blood viscosity and the microrheological parameters are presented for the patients with ET. One can see that there are certain differences for the correlation coefficients between RBC aggregation and RBC deformation properties, viscosity and microrheological properties, etc. Interestingly, the whole blood viscosity at the high shear rate (1000 s^{-1}) positively correlate with the AI parameter ($r = 0.85$) and negatively correlate with time parameters of RBC aggregation ($T_{1/2}$, τ_1, τ_2) ($r < -0.55$). Also, negative correlation between blood viscosity and RBC deformation can be observed via Spearman's rank correlation coefficients (Fig. 6). This means that the lower the deformability of RBCs, the higher the blood viscosity, which is consistent with the general concept [1].

The weak positive correlation between CSS and AI parameters is observed ($p = 0.36$), whilst the high positive correlation between CSS and M parameters is observed ($p = 0.75$) (Fig. 6). It means that in patients with ET, the higher the aggregation, the higher the hydrodynamic strength of the RBC aggregates. In other words, it means that the processes of RBC aggregation and disaggregation are positively correlated with each other. It should be noted that the M and AI parameters are calculated almost in the same way, except that the AI parameter is normalized, and the M parameter is not (Fig. 1). However, considering correlation matrix, one can see that M and AI parameters are not correlated ($p = -0.12$).

The parameter of normalized whole blood viscosity (η_0) is correlated with microrheological parameters almost the same as the whole blood viscosity (η). This probably means that plasma composition is a major factor affecting changes in whole blood viscosity at high shear stresses in patients with ET.

In the future, with large statistical magnification such correlation analysis between the parameters could be used in diagnosis or therapy monitoring. In this pilot study, due to the limitations imposed by the small sample group sizes, it is not feasible to draw broad conclusions.

This study has several limitations. First, there is no control group due to the lack of healthy people of approximately the same age as the patient. However, the results obtained from the small sample group size still provide preliminary conclusions on the relationships between whole blood viscosity and microrheological parameters for patients with ET. It is evident that the inclusion of more data from larger study groups will help reduce statistical scattering and yield more accurate information on the correlations being studied. Additionally, it is important to note that only patients aged 8–17 participated in this study. Increasing the number of individuals in the study groups will allow for the identification of any alterations in the studied parameters and their correlations, considering such factors as the age and the specific diseases being studied.

4 Conclusions

This pilot study has shed light on the correlation between whole blood viscosity and microrheological parameters in the context of blood disorders. The novel data presented have provided valuable preliminary evidence for better understanding the relationship between blood viscosity and microrheological parameters of patients with ET as well as between different microrheological parameters of patients with ET, ERY, and SPH. However, it is important to consider the limitations of this study, such as the small sample group size and lack of control groups. Therefore, further research with larger sample group sizes and control groups is necessary to validate these findings and determine the potential diagnostic and treatment implications for myeloproliferative disorders and SPH.

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Disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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