agonist romiplostim might be effective for treating thrombocytopenia in SGA infants.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

ST designed the study, preformed all experiments, analyzed and interpreted the data and wrote the manuscript. HK collected and assembled the data, performed data analysis and interpretation and wrote the manuscript. KT, HA, HU and YY collected and assembled the data and performed data analysis and interpretation. MA designed the study, analyzed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Primer pairs used for polymerase chain reaction amplification.

Table SII. Blood cell counts of control and SGA rats.

Figure S1. Body weight of control and SGA rats.

Figure S2. *Tpo* expression in tissues of control and SGA rats.

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Impact of COVID-19 on red blood cell rheology

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory coronavirus 2 (SARS-CoV-2). Among hospitalized patients, many have developed typical manifestations of sepsis-like cold extremities, weak peripheral pulses or severe metabolic acidosis, indicating microcirculation dysfunction.[1] Microvascular dysfunction has been reported in sepsis[2,3] and could be partly caused by red blood cell (RBC) rheological alterations such as decreased RBC deformability

Table I. General characteristics and biological parameters in the different groups.

		COVID (n = 7)	SEPSIS (n = 7)	CONTROL (n = 7)
Admission medical check	Age	71.3 [37.4; 74.3]	**79.2 [61.8; 81.7]‡**	56.1 [32.7; 59.8]
	Sex (M/F)	5/2	6/1	3/4
	Weight (kg)	57.5 [41.0; 80.0]	66.0 [63.0; 89.0]	NA
	Height (cm)	162.0 [152.0; 164.0]	172.0 [171.0; 174.0]	NA
	BMI (kg/m^2)	25.7 [18.3; 26.5]	21.8 [21.5; 30.1]	NA
	CF	86.0 [64.0; 110.0]	90.0 [81.0; 94.0]	NA
	SBP (mm Hg)	130.0 [120.0;167.0]	134 [130.0; 166.0]	NA
	DBP (mm Hg)	78.0 [61.0; 80.0]	79.0 [71.0; 85.0]	NA
	Fever (n)	2	1	NA
Medical history	Obesity (n)	2	3	0
	Smokers (n)	0	1	1
	HBP (n)	2	2	0
	Diabetes (n)	2	3	0
	Cardiac failure (n)	3	2	0
	Hepatic failure (n)	0	1	0
	Renal failure (n)	4	4	0
	Metabolic syndrome (n)	4	1	0
	Cancer (n)	0	1	0
Biological parameters	WBC (G/l)	**5.6 [3.5; 9.8]***	12.8 [9.6; 15.9]	NA
	RBC (T/l)	4.3 [3.2; 5.2]	3.3 [3.1; 4.5]	NA
	Hb (g/l)	116.0 [90.0; 144.0]	107.0 [98.0; 124.0]	NA
	Hct (%)	39.0 [30.0; 43.0]	36.0 [33.0; 40.0]	43.0 [39.5; 46.5]
	MCV (fl)	89.4 [81.3; 94.0]	92.9 [88.2; 100.9]	NA
	MCHC (g/l)	324.0 [313.0; 334.0]	324.0 [321.0; 332.0]	NA
	PLT (G/l)	212.0 [153.0; 390.0]	289.0 [178.0; 401.0]	NA
	CRP (mg/l)	**94.6 [74.1; 161.6]***	205.3 [182.8; 241.4]	NA
	ASAT (UI/l)	33.5 [23.0; 57.0]	39.0 [33.0; 76.0]	NA
	ALAT (UI/l)	18.0 [11.0; 24.0]	26.0 [15.0; 31.0]	NA
	Fibrinogen (g/l)	6.4 [5.5; 7.6]	5.8 [4.7; 6.3]	NA
	P50	29.5 [27.2; 31.0]	26.7 [26.3; 29.3]	27.6 [27.0; 30.6]
Haemorheological parameters	RBCD 0·3 Pa (a.u.)	0.065 [0.047; 0.079]	0.076 [0.057; 0.101]	0.064 [0.056; 0.068]
	RBCD 0·53 Pa (a.u.)	**0.131 [0.092; 0.138]***	0.154 [0.130; 0.165]	0.136 [0.129; 0.136]
	RBCD 0·95 Pa (a.u.)	**0.200 [0.162; 0.219]***	0.234 [0.209; 0.242]	0.216 [0.207; 0.219]
	RBCD 1·69 Pa (a.u.)	**0.282 [0.241; 0.300]*,†**	0.305 [0.297; 0.324]	0.297 [0.288; 0.303]
	RBCD 3 Pa (a.u.)	**0.374 [0.330; 0.384]*,†**	0.384 [0.378; 0.403]	0.380 [0.374; 0.389]
	RBCD 5·33 Pa (a.u.)	**0.448 [0.410; 0.454]†**	0.454 [0.441; 0.467]	0.454 [0.449; 0.459]
	RBCD 9·49 Pa (a.u.)	**0.500 [0.473; 0.510]†**	0.503 [0.489; 0.512]	0.510 [0.502; 0.517]
	RBCD 16·87 Pa (a.u.)	**0.541 [0.513; 0.552]†**	0.542 [0.528; 0.552]	0.551 [0.545; 0.562]
	RBCD 30 Pa (a.u.)	**0.579 [0.544; 0.587]†**	**0.574 [0.561; 0.586]‡**	0.588 [0.578; 0.598]
	Maximum RBCD (a.u.)	**0.64 [0.58; 0.65]†**	**0.63 [0.62; 0.64]‡**	0.67 [0.65; 0.68]
	Blood viscosity (cP)	5.8 [4.9; 6.0]	5.1 [4.2; 6.1]	5.5 [4.8; 6.0]
	RBC aggregation			
	M (a.u.)	**11.9 [8.3; 13.5]*,†**	3.6 [2.4; 9.3]	6.8 [6.4; 9.8]
	M1 (a.u.)	**14.6 [12.5; 19.6]†**	**13.5 [13.1; 16.3]‡**	10.2 [9.4; 12.0]
Outcome	Medical care	4	7	NA
	Intensive care	3	0	NA
	Death	0	0	NA

A non-linear curve fitting was applied on the shear stress red blood cell deformability (RBCD) curves to determine the maximum RBCD in each group.[15] Results are presented as median [25th–75th] percentiles. Chi-squared test was used to compare the distribution of gender between groups. A non-parametric Kruskal–Wallis analysis of variance followed by Dunn post-hoc tests were used for the comparisons of three groups, while a Mann–Whitney U test was used when data were available in SEPSIS and COVID groups only. BMI, body mass index; CF, cardiac frequency; SBP, systolic blood pressure; DBP, diastolic blood pressure; HBP, high blood pressure; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; PLQ, platelets; CRP, C-reactive protein; ASAT, aspartate amino transferase; ALAT, alanine amino transferase; P50, oxygen tension at which 50% of Hb is saturated with oxygen; a.u., arbitrary units; M, RBC aggregation at stasis; M1, RBC aggregation at a low shear rate (3 s^{-1}); NA, not available. Significance level was defined as $P < 0.05$. Significant values are highlighted in bold. COVID *versus* SEPSIS group: *$P < 0.05$; COVID *versus* CONTROL group: †$P < 0.05$; SEPSIS *versus* CONTROL group: ‡$P < 0.05$.

(RBCD),[4] increased RBC aggregation[5] and abnormal RBC morphology/physiology.[6] Whether COVID-19 patients also have impaired RBC rheology is unknown. The aim of this preliminary study was to compare the haemorheological profile (RBCD, RBC aggregation, blood viscosity) between COVID-19 patients, sepsis patients and healthy controls.

Material and methods

Seven COVID-19 patients (COVID) hospitalized in Edouard-Herriot Hospital (Hospices Civils de Lyon, Lyon, France) were included. COVID-19 diagnosis was made by specific real-time polymerase chain reaction (RT-PCR; $n = 5$) and/or by typical chest computerized tomography ($n = 7$). Seven patients admitted for non-COVID-related sepsis (SEPSIS) and seven healthy controls (CONTROL) were also included. SEPSIS patients were identified by the quick Sequential Organ Failure Assessment (quickSOFA) score according to Sepsis-3 definition.[7] Clinical reports were reviewed by a physician to collect general and clinical characteristics (Table I). The study was approved by the local ethics committee (No. 20-108) and the Hospices Civils de Lyon biological resource centre (CRB HCL, BB-0033-00046).

Haematological parameters were determined using a haematology analyser (XN-9000; Sysmex Corporation, Kobe, Japan) for COVID and SEPSIS patients. The haemorheological parameters (RBCD, RBC aggregation and blood viscosity) were measured by ektacytometry, Myrenne aggregometer and cone/plate viscometer respectively, as previously described.[8] The Hemox-Analyzer (TCS, Medical Products Division, Southampton, PA, USA) was used to plot the oxygen haemoglobin dissociation curves and determine the P50 values (the oxygen tension at which 50% of Hb is saturated with oxygen), with adjustment of pH at 7.4 and temperature at 37°C.[9]

Results

Gender distribution was not different between the three groups (Table I). The age of the SEPSIS group was significantly higher than the CONTROL group ($P < 0.05$). No other differences in age between groups were detected. In the COVID group, none of the patients were smokers, but every patient had co-morbidities such as obesity, high blood pressure, diabetes, metabolic syndrome, cardiac, hepatic or renal failure. COVID patients were globally severe with marked lung alterations, as evidenced by the extent of parenchymal lesions (>50% in 43% of cases; data not shown) and a transfer to intensive care unit (ICU) for three of them (none in the SEPSIS group). No patient in any group died during the study.

White blood cell counts and C-reactive protein were significantly lower in the COVID than in the SEPSIS group ($P < 0.05$). The other common biological parameters did not differ between these two groups. There were no differences in P50 values between groups. RBCD was significantly lower in the COVID group compared to the SEPSIS group at low shear stresses (i.e., from 0.53 to 3 Pa; $P < 0.05$). In addition, RBCD from COVID patients was significantly lower than the CONTROL group for shear stresses, ranging from 1.69 to 30 Pa ($P < 0.05$). RBCD was also significantly reduced in the SEPSIS compared to the CONTROL group at 30 Pa ($P < 0.05$). Maximum RBCD was reduced in both COVID and SEPSIS groups compared to the CONTROL group ($P < 0.05$). Blood viscosity was not significantly different between the three groups. No difference in fibrinogen levels was shown between COVID and SEPSIS patients. Despite no data available in the CONTROL group, the concentrations were above the normal range (reference values of our laboratory: 2–4 g/l). RBC aggregation measured at stasis was significantly higher in the COVID group compared to the two other groups, while RBC aggregation measured at a low shear rate was significantly higher in both the COVID and SEPSIS groups compared to the CONTROL group.

Discussion

Our results showed that in COVID-19 patients, RBC aggregation is increased both at stasis and at a low shear rate, compared to CONTROL individuals, while RBC aggregation in SEPSIS patients was higher than healthy individuals at low shear rate only. RBC aggregation at a low shear rate, in comparison to a static condition, increases the chance of RBCs to make contact and aggregate, which would better reflect *in-vivo* conditions. It is possible that the high fibrinogen levels observed in both COVID and SEPSIS patients, relative to the 2–4 g/l reference value used in our laboratory, was a contributing factor to increased RBC aggregation,[10] but further work is needed to test the contribution of RBC aggregability (i.e., cellular factors). Clot stability has been shown to be affected by the RBC rheological properties.[11] Indeed, enhanced RBC aggregation could affect clot structure and increase their resistance. The lack of peripheral blood smears in the present study did not allow us to clearly differentiate RBC aggregation from RBC agglutination, which may also be a factor in COVID-19 pathophysiology.

The present study also showed a decrease in RBCD in the COVID group compared to the CONTROL group at shear stresses ranging from 0.53 to 30 Pa. Changes in RBC membrane lipids composition and RBC membrane protein fragmentation could explain the RBCD reduction in COVID-19 patients.[12] RBCD at 30 Pa and maximum RBCD were also different between the CONTROL and SEPSIS groups, suggesting that RBC from sepsis patients would have increased internal viscosity and/or a loss in the surface/volume ratio, but not impaired membrane elasticity, in contrast to COVID-19 patients.[13] Despite the differences in RBC rheological properties, no difference in blood viscosity was observed between the three groups, possibly because haematocrit tended to be decreased in the COVID and SEPSIS groups.

The lack of difference in measured P50 between COVID-19 and healthy individuals contrasts with a recent study

where P50 was calculated from blood gas analyzer.[14] Our results suggest that SARS-CoV-2 does not affect Hb oxygen affinity directly, but COVID-19 patients may have increased Hb oxygen affinity due to hypoxia/metabolic adaptations.[14]

Conclusion

In conclusion, this preliminary study shows that RBC rheological properties are impaired in COVID-19 patients. This could increase the risks for thromboembolic events, as well as affect microvascular blood flow. Nevertheless, the sample size of this study was limited and further studies are needed.

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Author contributions

Céline Renoux, Romain Fort, Elie Nader, Philippe Joly, Emeric Stauffer, Mélanie Robert, Agnès Cibie, Alexandra Gauthier and Philippe Connes designed the research study. Romain Fort, Céline Renoux and Sandrine Girard included patients or controls. Camille Boisson, Elie Nader and Philippe Joly performed the research. Céline Renoux, Romain Fort, Elie Nader, Philippe Joly and Philippe Connes analysed the data. Céline Renoux, Romain Fort, Elie Nader and Philippe Connes wrote the paper. All the authors revised the paper critically and approved the final version.

Conflict of interest

The authors declare no conflicts of interest.

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