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Evidence of systemic endothelial injury and microthrombosis in hospitalized COVID-19 patients at different stages of the disease

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Highlights

- Schistocytes are fragments of red blood cells which may be encountered in the peripheral blood smear of patients suffering from a variety of microangiopathic diseases.
- In the hospitalized COVID-19 patients at different stages of disease severity, a schistocyte count ≥ 1% was documented in approximately 70% of patients.
- Evidence of myocardial injury was observed in 87.5% of all who had a count of schistocytes ≥ 1%.
- Schistocytes may serve as a simple and inexpensive biomarker to identify a high-risk subpopulation with a latent systemic microvascular damage irrespective of respiratory symptoms.

Severe endothelial injury and widespread microthrombosis have been recently described in postmortem examinations of coronavirus disease-2019 (COVID-19) patients [1–5].

Whether a systemic microangiopathy is present at different stages of the disease irrespective of the extent of pulmonary involvement, it has not been confirmed.

Yet, a wide range of extrapulmonary clinical manifestations (e.g. thromboembolism, myocardial infarction with normal coronary arteries, kidney function impairment) has been reported in a significant number of COVID-19 patients [6]. Such manifestations usually accompany the most common respiratory symptoms, even if they appear to be unrelated to the severity of lung involvement, and may manifest at any stage of the disease, therefore suggesting the presence of an underlying systemic vascular disorder with hypercoagulability as a common pathophysiological substrate of the disease.

Schistocytes are fragments of red blood cells which may be encountered in the peripheral blood smear (PBS) of patients suffering from a variety of microangiopathic diseases [e.g. disseminated intravascular coagulation (DIC), thrombocytopenic purpura].

They are the result of a mechanical damage to erythrocytes which are sheared by fibrin strands of microthrombi in the peripheral circulation. The underlying pro-thrombotic state is linked to a damage of the endothelium which promotes thrombus formation and microvascular dysfunction.

In this context, the presence of schistocytes may serve as a surrogate biomarker for in-vivo assessment of a diffuse endothelial damage with formation of fibrin thrombi.

We aimed at documenting the presence of schistocytes in the PBS of hospitalized COVID-19 patients at different stages of disease severity.

Fourteen consecutive patients with severe acute respiratory syndrome coronavirus-2 infection confirmed by reverse-transcriptase-polymerase-chain-reaction were included in this study. The median age was 70 years (IQR: 59–76) and 85.7% were males. Baseline characteristics are reported in Table 1.

PBSs were taken after a median of 3 days from admission (range 1–5 d) and examined by two experienced pathologists who were blinded to disease severity. The presence of schistocytes (abnormal cut-off value ≥ 1%) was microscopically evaluated following the International Council for Standardization in Hematology recommendations [7].

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Table 1 Baseline characteristics, laboratory results, drug therapy, and outcomes of the overall population (Panel A), and characteristics of 7 patients requiring mechanical ventilation (Panel B) and of 7 patients under noninvasive ventilation or high-flow nasal cannula (Panel C)

Characteristic	Overall (n = 14)	Invasive Mechanical Ventilation (n = 7)	Noninvasive Ventilation/ Nasal Cannula (n = 7)
Panel A			
Age, median (IQR) [yr]	70 (59–76)	70 (58–76)	70 (60–75)
Male Sex, n (%)	12 (85.7)	7 (100)	5 (71.4)
Medical History			
Hypertension, n (%)	10 (71.4)	5 (71.4)	5 (71.4)
Diabetes, n (%)	8 (57.1	5 (71.4)	3 (42.9)
Symptoms at Onset			
Fever, n (%)	13 (92.9)	7 (100)	6 (85.7)
Cough, n (%)	11 (78.6)	5 (71.4)	6 (85.7)
Diarrhea, n (%)	3 (21.4)	1 (14.3)	2 (28.6)
Imaging Features			
Ground-Glass Opacity, n (%)	13 (92.9)	7 (100)	6 (85.7)
Bilateral Pulmonary Infiltrates, n (%)	14 (100)	7 (100)	7 (100)
Laboratory Findings			
White-Cell Count/mm^3, median (IQR)	6,740 (5,880–10,900)	6,870 (5,790–12,600)	6,200 (5,950–9,050)
White-Cell Count/$mm^3 > 10{,}000$, n (%)	4 (28.6)	3 (42.9)	1 (14.3)
Lymphocytes Count/mm^3, median (IQR)	750 (667–1,052)	790 (685–1,140)	700 (545–950)
Lymphocytes Count/$mm^3 < 1{,}000$, n (%)	9 (64.3)	4 (57.1)	5 (71.4)
Platelet Count/mm^3, median (IQR)	196,000 (150,000–245,000)	163,000 (106,000–196,000)	229,000 (193,000–341,00)
Platelet Count/$mm^3 < 100{,}000$, n (%)	2 (14.3)	2 (28.6)	0 (0.0)
LDH, median (IQR) [U/L]	370 (284–531)	295 (278–519)	398 (353–423)
LDH > 280 U/L, n (%)	10 (71.4)	4 (57.1)	6 (85.7)
Creatinine, median (IQR) [μg/L]	97 (83–135)	84 (82–103)	117 (97–146)
PT, median (IQR) [sec]	12.8 (12.3–16.8)	12.4 (11.9–13.8)	15.0 (12.7–29.0)
aPTT, median (IQR) [sec]	33.3 (31.5–35.8)	34.1 (32.3–48.9)	32.0 (29.9–35.4)
Fibrinogen, median (IQR) [g/L]	4.6 (2.8–7.26)	2.9 (1.6–5.1)	6.3 (4.6–7.9)
Fibrinogen < 1 g/L, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
D-dimer, median (IQR) [mg/L]	1.91 (1.26–4.47)	2.21 (1.81–4.47)	1.21 (0.91–3.21)
D-dimer > 1 mg/L, n (%)	11 (78.6)	7 (100)	4 (57.1)
Peripheral Blood Smear			
Schistocyte > 1%, n (%)	10 (71.4)	5 (71.4)	5 (71.4)
Treatment			
Antibiotic Agent, n (%)	13 (92.9)	6 (85.7)	7 (100)
Antiviral Agent, n (%)	1 (7.1)	1 (14.3)	0 (0.0)
Hydroxychloroquine, n (%)	13 (92.9)	6 (85.7)	7 (100)
Corticosteroids, n (%)	4 (28.6)	1 (14.3)	3 (42.9)
Outcomes			
Death, n (%)	3 (21.4)	2 (28.6)	1 (14.3)

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Panel B							
Age, yr	72	82	70	54	38	81	62
Sex	Male	Male	Male	Male	Male	Male	Male
Initial findings							
Medical history	Hypertension Diabetes Hyperthyroidism	Hypertension Diabetes Alzheimer	Hypertension	Hypertension Diabetes	Asthma	Hypertension Diabetes	Diabetes

Table 1 (continued)

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Symptoms at disease onset	Fever Dyspnea Cough Diarrhea	Fever Dyspnea Syncope	Fever Dyspnea Cough	Fever Dyspnea Cough	Fever Dyspnea Cough	Fever Dyspnea Cough	Fewer Dyspnea Asthenia
Imaging features	Ground-glass opacity, Bilateral pulmonary infiltrates	Ground-glass opacity, Bilateral pulmonary infiltrates	Ground-glass opacity, Bilateral pulmonary infiltrates	Ground-glass opacity, Bilateral pulmonary infiltrates	Ground-glass opacity, Bilateral pulmonary infiltrates	Ground-glass opacity, Bilateral pulmonary infiltrates	Ground-glass opacity, Bilateral pulmonary infiltrates
Treatment	Hydroxychloroquine Antibiotics	Hydroxychloroquine Lopinavir-Ritonavir	Hydroxychloroquine Immune Globulin Antibiotics	Hydroxychloroquine Antibiotics	Immune Globulin Antibiotics	Hydroxychloroquine Immune Globulin Antibiotics	Hydroxychloroquine Immune Globulin Antibiotics
Laboratory findings							
White cell count (per mm3)	6,610	23,290	4,970	6,870	13,740	11,470	3,920
Total Neutrophils (per mm3)	5,370	21,800	3,740	4,750	11,910	7,210	3,070
Total Lymphocytes (per mm3)	710	790	660	1,210	1,070	3,670	330
Total monocytes (per mm3)	270	260	400	320	540	340	360
Platelet count (per mm3)	217,000	361,000	163,000	140,000	65,000	175,000	75,000
Hemoglobin (g/L)	88	103	137	88	112	89	83
Albumin (g/L)	25	26	34	36	30	26	27
Alanine aminotransferase (U/L)	23	25	22	14	72	62	22
Aspartate aminotransferase (U/L)	20	40	35	12	5	25	9
Lactate dehydrogenase (U/L)	249	295	280	383	1,433	654	276
Creatinine (mg/dL)	1.55	1.10	0.81	0.71	0.84	0.95	0.83
High-sensitivity cardiac troponin T (μg/L)	0.042	0.073	0.010	0.009	0.033	0.009	0.046
Prothrombin time (sec)	11.07	12.41	12.34	11.49	14.93	12.75	26.62

Table 1 (continued)

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Activated partial thromboplastin time (sec)	34.1	32.1	71.0	35.6	29.6	32.5	62.2
Fibrinogen (g/L)	8.39	6.67	1.96	3.61	1.18	2.88	1.12
D-Dimer (mg/L)	4.473	2.217	1.886	1.430	4.474	4.502	1.732
Procalcitonin (ng/mL)	0.04	0.88	0.05	0.18	0.16	0.22	0.023
High-sensitivity C-reactive protein (mg/L)	3.3	318	1.4	27.5	3.2	10.9	46.4
Peripheral blood smear							
Schistocyte (%)	1–2	1–2	1–2	1–2	0.5	0.5	1–2

Characteristic	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14
Panel C							
Age, yr	75	82	70	76	42	63	58
Sex	Male	Male	Female	Male	Male	Female	Male
Initial findings							
Medical history	Hypertension	Hypertension COPD	Hypertension Diabetes	Hypertension Diabetes	–	Hypertension Diabetes	–
Symptoms at disease onset	Fever Dyspnea Cough Diarrhea	Fever Dyspnea Cough Diarrhea	Fever Dyspnea Cough	Dyspnea Cough	Fever Dyspnea Cough	Fever Dyspnea Cough	Fever Dyspnea
Imaging features	Ground-glass opacity, bilateral pulmonary infiltrates	Ground-glass opacity, bilateral pulmonary infiltrates	Focal airspace disease in the peripheral right midlung, discoid atelectasis at the left lung base	Ground-glass opacity, bilateral pulmonary infiltrates	Ground-glass opacity, bilateral pulmonary infiltrates	Ground-glass opacity, bilateral pulmonary infiltrates	Ground-glass opacity, bilateral pulmonary infiltrates
Treatment	Hydroxychloroquine immune globulin antibiotics	Hydroxychloroquine immune globulin antibiotics	Hydroxychloroquine antibiotics	Hydroxychloroquine antibiotics	Hydroxychloroquine antibiotics	Hydroxychloroquine antibiotics	Hydroxychloroquine antibiotics
Laboratory findings							
White cell count (per mm3)	2,750	5,800	8,830	6,250	11,670	6,120	9,380
Total neutrophils (per mm3)	1,470	5,010	7,400	7,500	2,000	–	8,200
Total lymphocytes (per mm3)	690	350	900	400	700	1,000	1,500
Total monocytes (per mm3)	410	270	300	500	400	200	200

Table 1 (continued)

Characteristic	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14
Platelet count (per mm3)	146,000	165,000	430,000	229,000	221,000	251,000	531,000
Hemoglobin (g/L)	112	127	98	128	169	96	137
Albumin (g/L)	30	28	31	36	47	30	33
Alanine aminotransferase (U/L)	110	23	10	24	48	23	144
Aspartate aminotransferase (U/L)	151	12	20	59	75	47	36
Lactate dehydrogenase (U/liter)	357	398	241	348	506	677	539
Creatinine (mg/dL)	0.72	0.98	1.61	0.97	1.35	1.50	0.67
High-sensitivity cardiac troponin T (µg/L)	0.009	0.012	0.1	0.076	0.037	0.011	0.023
Prothrombin time (sec)	12.55	10.76	35.9	13.2	–	16.8	33.1
Activated partial thromboplastin time (sec)	29.9	32	–	36.2	35.4	–	13.2
Fibrinogen (g/L)	2.80	4.26	7.46	6.27	4.97	8.39	8.59
D-Dimer (mg/L)	0.909	4.481	1.940	0.920	1.210	4.930	0.810
Procalcitonin (ng/mL)	0.08	0.08	0.5	0.1	0.09	0.3	0.01
High-sensitivity C-reactive protein (mg/L)	4.1	76.9	1.4	27.5	3.2	10.9	46.4
Peripheral blood smear							
Schistocyte (%)	0.5–1	0	1–2	1–2	1–2	1–2	1–2

aPTT Activated partial thromboplastin time, *IQR* interquartile range, *LDH* lactate dehydrogenase, *PT* prothrombin

Patients with mechanical cardiac valvular prostheses, chronic kidney disease stage 4–5, diabetic microangiopathy, or other causes of schistocyte formation were excluded. None had required hemodialysis or extracorporeal membrane oxygenation during hospitalization.

Symptoms and signs at presentation included: fever (93%), cough (79%), and diarrhea (21%).

At the time PBS was performed, patients were hospitalized and had different degrees of COVID-19 severity: 7 (50%) patients had severe lung injury requiring invasive mechanical ventilation, 2 (14.3%) noninvasive ventilation, and 5 (35.7%) high-flow nasal cannula.

A schistocyte count ≥ 1% was documented in 10 (71.4%) patients; one (7.1%) patient had 0.8% and 3 (21.4%) had ≤ 0.5%.

The median platelet count was 196,000/mm^3 (IQR: 150,000-245,000) and all but 2 (14.3%) patients had > 100,000 platelets/mm^3.

None had a fibrinogen level < 1 g/L and fulfilled the diagnostic criteria for overt DIC.

Evidence of myocardial injury, as demonstrated by elevated levels of high-sensitive troponin T (> 0.014 µg/L), was observed in 8 (57.1%) patients, 7 of whom (87.5%) had a count of schistocytes ≥ 1% and no preexisting history of cardiovascular disease. All 8 patients had normal left ventricular ejection fraction (EF) but one (patient 2) with reduced EF and regional wall motion abnormalities.

All patients were prescribed with systemic anticoagulation, 4 (28.6%) received low-dose corticosteroids.

During hospitalization, one patient had pulmonary thromboembolism (patient 10) and 3 (21.4%) died of multiorgan failure. All four patients had a count of schistocytes > 1%. At the time of discharge, PBS was repeated in other 4 patients with a previous abnormal schistocyte value [after a median of 22 days (range 16–28)], revealing a normal count in all.

Hereby, we report a high prevalence (71.4%) of an abnormal count of schistocytes in the PBS of COVID-19 patients. Schistocytes were observed at any stage of disease severity, irrespective of lung involvement. Additionally, increased high-sensitive troponin T was observed in the majority of patients with schistocytes (7 out of 10; 70.0%), compared to those without.

Since none of the patients fulfilled the diagnostic criteria for overt DIC and other causes of schistocyte formation were excluded, the presence of these fragments of red blood cells may imply a subclinical impairment of the endothelial cell layer of the microvasculature with formation of microthrombi in the coronary and peripheral circulation.

These findings are consistent with recent studies which described endotheliitis and a systemic microthrombotic disease in patients who died from COVID-19 [1, 3]. However, our study for the first time extends the observations of postmortem studies by correlating a similar pathophysiological substrate also to milder forms of the disease.

This pattern of endothelial injury and hypercoagulability may explain the variety of clinical manifestations (e.g. kidney failure, myocardial infarction with normal coronary arteries, neurological manifestations, purpura) that has been described so far in COVID-19 patients [6].

As such, a therapeutic approach targeting the underlying endothelial dysfunction and prothrombotic state (e.g. early systemic anticoagulation, immunomodulators) may be justified at any stage of the disease to prevent clinical progression and multi-organ involvement.

Additionally, schistocytes may serve as a simple and inexpensive biomarker to identify a high-risk subpopulation with a latent systemic microvascular damage irrespective of respiratory symptoms.

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Compliance with ethical standards

Conflict of interest All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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