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Curative anticoagulation prevents endothelial lesion in COVID-19 patients

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Abstract

Background: Coronavirus disease-2019 (COVID-19) has been associated with cardiovascular complications and coagulation disorders.

Objectives: To explore the coagulopathy and endothelial dysfunction in COVID-19 patients.

Methods: The study analyzed clinical and biological profiles of patients with suspected COVID-19 infection at admission, including hemostasis tests and quantification of circulating endothelial cells (CECs).

Results: Among 96 consecutive COVID-19-suspected patients fulfilling criteria for hospitalization, 66 were tested positive for SARS-CoV-2. COVID-19-positive patients were more likely to present with fever (P = .02), cough (P = .03), and pneumonia at computed tomography (CT) scan (P = .002) at admission. Prevalence of D-dimer >500 ng/mL was higher in COVID-19-positive patients (74.2% versus 43.3%; P = .007). No sign of disseminated intravascular coagulation were identified. Adding D-dimers >500 ng/mL to gender and pneumonia at CT scan in receiver operating characteristic curve analysis significantly increased area under the curve for COVID-19 diagnosis. COVID-19-positive patients had significantly more CECs at admission (P = .008) than COVID-19-negative ones. COVID-19-positive patients treated with curative anticoagulant prior to admission had fewer CECs (P = .02) than those without. Interestingly, patients...
In December 2019, an epidemic pneumonia caused by a new coronavirus occurred in Wuhan (Hubei Province) and spread rapidly throughout China, evolving into a global pandemic. Originally called new coronavirus 2019 (2019-nCoV), this virus was then officially named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the World Health Organization (WHO). On January 30, 2020, the WHO declared the SARS-CoV-2 epidemic (COVID-19) a public health emergency of international concern. Compared to SARS-CoV, which caused a severe acute respiratory syndrome (SARS) epidemic in 2003, SARS-CoV-2 has a higher transmission capacity with a higher mortality. With a rapid increase in confirmed cases, there is an unmet need of prevention and therapeutic strategies of COVID-19. Although COVID-19’s clinical manifestations are dominated by respiratory symptoms, some patients have severe cardiovascular damage and kidney disease contributing to multiple organ failure. Patients with cardiovascular comorbidities may also be at higher risk of death. For example, COVID-19 patients with hypertension have an increased mortality and morbidity with hazard ratio
ranging from 1.7 to 3.05, depending on the study. Based on this, rising concerns have emerged regarding angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs). Indeed, angiotensin-converting enzyme 2 (ACE2) has been shown to be a co-receptor for viral entry for SARS-CoV-2, and it has been demonstrated that ACEi and ARBs could enhance ACE2 expression, which may therefore jeopardize patient susceptibility to viral host cell entry and dissemination.

During the epidemic in China, coagulopathy was reported in severe COVID-19 patients. D-dimer levels above 1000 ng/mL were an independent risk factor of in-hospital death. Coagulopathy was also found in fatal cases of COVID-19 patients, including a significantly higher proportion of patients with D-dimers above 500 ng/mL and prolonged prothrombin time (PT) in non-survivors. This study was replicated in a second Chinese population in which D-dimers were still associated with in-hospital mortality. The hypothesis of microthrombi in kidneys was also suggested in COVID-19 patients because high creatinine level was correlated with D-dimers above 500 ng/mL. Finally, endothelial dysfunction might also play a role in the incidence of severe respiratory symptoms and viral systemic dissemination. Indeed, the SARS-CoV-2 receptor ACE2 is strongly expressed on endothelial cells. We hypothesized that endothelial cell infection may induce endothelium damage and dysfunction/activation that triggers coagulation activation. Circulating endothelial cells (CECs) are considered relevant markers of endothelial lesion or dysfunction and were used to explore the potential vascular dysfunction in COVID-19 patients.

The aim of our study was to identify biological markers related to COVID-19 diagnosis and severity. We also aimed at better characterizing subpopulations of patients at risk of coagulopathy and/or endothelial dysfunction to target COVID-19 patients at risk for the worst outcomes.

2 | METHODS

2.1 | Study design and population

This is a single-center, prospective observational cohort study conducted in a university hospital in Paris (France). From March 14, 2020 to March 20, 2020, all consecutive patients aged over 18 years, presenting to the emergency room of the Georges Pompidou European hospital and fulfilling hospitalization criteria or direct in-patient referral, with an infectious syndrome suspect of COVID-19 were included. Hospitalization criteria were based on local guidelines as described in Table 1. Suspicion of COVID-19 was defined by the presence of at least one of the following: fever, headache, myalgia, cough, dyspnea, rhinorrhea, or digestive symptoms. All COVID-19-suspected patients had a clinical evaluation, blood tests, and computed tomography (CT) scan, and were tested for SARS-CoV-2 infection by nasopharyngeal swab before they were transferred to dedicated hospitalization units: medical department or intensive care unit (ICU). The study was performed in accordance with the Declaration of Helsinki. All patients provided written informed consent before enrollment (CPP 2020-04-048/ 2020-A01048-31/ 20.04.21.49318). For all patients, baseline characteristics (demographic, treatment, clinical, cardiovascular risk factors, and body mass index), biological data, and CT scan results were retrieved from the medical records using standardized data collection.

2.2 | Laboratory confirmation of SARS-CoV-2 infection

Nasopharyngeal swabs were collected in universal transport medium (Xpert® nasopharyngeal sample collection kit). SARS-CoV-2 was detected using Allplex™ 2019-nCoV Assay (Seegene), a multiplex real-time polymerase chain reaction (PCR) assay that detects three target genes (E gene, RdRP gene, and N gene) in a single tube. Data were automatically analyzed using Seegene viewer software. Only qualitative data were available.

2.3 | Routine blood examinations

All samples were collected on ethylenediaminetetraacetic acid (EDTA), sodium heparin, and 0.129 M trisodium citrate tubes (9NC BD Vacutainer, Plymouth, UK). Routine lab tests were complete blood count, creatinine, C-reactive protein (CRP), and high-sensitive troponin I (hs-TnI). Coagulation tests were PT ratio, fibrinogen, soluble fibrin monomer (STA®-Liatest FM; Stago), and antithrombin levels (Stachrom® AT III 6, Stago) explored on a STA-R® Max (Stago) coagulometer as previously described. D-dimers concentrations were determined using the Vidas D-Dimer assay (BioMérieux) according to the manufacturer’s instructions.

2.4 | CECs quantification

Peripheral venous blood samples were collected on EDTA after having always discarded the first milliliter of blood to avoid presence of endothelial cells dislodged by puncture. CECs were isolated by immunomagnetic separation with mAb CD146-coated beads and staining with the fluorescent probe acidin orange as previously described.
2.5 | Statistical analysis

Continuous data were expressed as median [interquartile range: (IQR)] and categorical data as proportion. In the univariate analysis, we determined differences in median using the unpaired t-test (Mann-Whitney U test) for continuous variables and in proportions we were using the Chi-square test or Fisher exact test if necessary. We generated receiver operating characteristics (ROC) curve with the regression models that included variables with a significant difference in the univariate analysis.\(^ {18,19}\) The model included gender, pneumonia at CT scan, and D-dimers above 500 ng/mL. This model helped assess the extent to which the level of D-dimers influenced the predictability of COVID-19 diagnosis. We compared area under the curve (AUC) of each ROC curve using the Delong test. All analyses were two-sided and a P-value of \( P < .05 \) was considered statistically significant. Statistical analysis was performed using R studio software (R Foundation for Statistical Computing).

3 | RESULTS

3.1 | High level of D-dimers is a discriminant factor during COVID-19 suspicion

Among the 96 COVID-19-suspected patients included, 66 were positive for SARS-CoV-2. COVID-19-positive patients were more likely to be males (\( N = 44, 66.7\% \)) than COVID-19-negative patients (\( N = 13, 43.3\% ; P = .05 \)). Otherwise, the two populations were strictly comparable in terms of time from illness onset to hospital admission, age, body mass index, cardiovascular risk factors, medical history, and treatments (Table 2). Considering clinical features at admission, COVID-19-positive patients were more likely to have fever (\( P = .02 \)), cough (\( P = .03 \)), and interstitial pneumonia at CT scan (\( P = .002 \)). In terms of biological features (Table 3) COVID-19-positive patients had a significantly lower white blood cell count, including neutrophil count (respectively, \( P = .008 \) and 0.02). Regarding hemostasis, the proportion of COVID-19-positive patients with D-dimers above 500 ng/mL was significantly higher (74.2% versus 43.3%; \( P = .007 \)). No difference in PT ratio or fibrin monomers was observed between groups. In the context of COVID-19-associated coagulopathy with high levels of D-dimers, fibrinogen, and CRP at admission, the low level of fibrin monomers and normal antithrombin levels allowed us to exclude a disseminated intravascular coagulation (DIC). When adding D-dimers above 500 ng/mL to gender and pneumonia at CT scan, ROC curve area (Figure 1) significantly increased from AUC 0.73 (95% confidence interval [CI] 0.61-0.85) to AUC 0.82 (95% CI 0.69-0.95; \( P = .02 \)), and confirm the relevance of D-dimers in diagnosis of COVID-19 at admission in hospital. The ROC curve with D-dimers above 500 ng/mL yielded a high sensitivity of 98.1% (95% CI 50.0-100.0), a low specificity of 28.6% (95% CI 21.0-85.8), a high positive predictive value of 77.9% (95% CI 65.3-90.0), and a high negative predictive value of 85.7% (95% CI 55.0-100.0).

3.2 | Anticoagulated COVID-19-positive patients have a significant lower CECs count

CECs were quantified as markers of endothelial lesion, using the reference method\(^ {15}\) as detailed in the Method section.\(^ {15}\) Using this assay, the upper limit of normal range at 10 CECs per mL of whole blood was previously determined and confirmed in several studies, including ours.\(^ {13,20-22}\) Among COVID-19-positive patients, 64% were above this threshold, suggesting a SARS-CoV-2-induced endothelial lesion. For comparison, COVID-19 negative population had fewer CECs (\( P = .008 \); Table 3) with only 27% above the normal range (\( P = .012 \)). Because the coagulopathy observed in COVID-19 patients could be related to this endothelial lesion, we analyzed whether anticoagulation could impact CEC level. Patients treated with curative anticoagulation prior to admission for any medical reason (83% for atrial fibrillation; 17% for venous thromboembolism) had indeed a lower CEC level than those without curative anticoagulation: 9 [8, 17] versus 24 [14, 42] CECs per mL (\( P = .02 \)), respectively (Figure 2). Interestingly, in patients treated with ACEI or ARBs the effect of curative anticoagulation on the CEC level was more pronounced with 10 [5, 14] versus 30 [17, 43] CECs per mL (\( P = .007 \)) in those without curative anticoagulation (Figure 2).

4 | DISCUSSION

The originality of this study was to evidence an endothelial lesion during SARS-CoV-2 infection, as witnessed by increased levels of CECs. Second, we show that this endothelial damage is thwarted by curative anticoagulation.

Several Chinese studies found that increased D-dimer level correlated with in-hospital mortality,\(^ {5,7,8}\) suggesting a COVID-19-associated DIC.\(^ {7,8,23}\) However, in our population, no overt DIC was diagnosed at admission. Indeed, patients had no significant thrombocytopenia, a normal PT ratio, and high fibrinogen levels. This was confirmed by a low level of fibrin monomers, which are early markers of DIC. DIC might nevertheless be involved in patient worsening and in particular for those with acute respiratory distress syndrome. Therefore, the increase of D-dimers largely reported in COVID-19 patients is probably not related to DIC but might reflect the microthrombi formation. Indeed, histopathological observations and imaging features of pulmonary lesions associated with SARS-CoV-2 revealed intra-alveolar fibrin deposit in pulmonary samples.\(^ {24}\) Moreover, a recent study using standardized protective ventilation settings in COVID-19 patients confirmed that oxygenation was severely compromised with a moderate alteration in the respiratory system compliance. Hypercapnia high prevalence led to the hypothesis of a large amount of ventilated/not perfused alveoli, which could reflect diffuse microthrombi in the pulmonary microvascular bed.\(^ {25}\) In renal disease associated to COVID-19, thrombotic lesions were proposed, because high D-dimers were more commonly observed in patients with elevated baseline serum creatinine.\(^ {9}\) In this context, the International Society on Thrombosis and Haemostasis (ISTH) has...
recently recommended measuring D-dimers, PT ratio, and platelet count in all COVID-19 patients to help stratify those who may benefit from hospitalization and a close monitoring.\textsuperscript{26} Therefore, ISTH, the American College of Cardiology, and the French Society for Vascular Medicine suggested the use of prophylactic anticoagulation with low weight molecular heparin (LMWH) for COVID-19 patients, in the absence of any contraindications.\textsuperscript{26-28} Indeed, preventive LMWH treatment could be associated with better prognosis in severe COVID-19 patients meeting sepsis-induced coagulopathy criteria.\textsuperscript{29}

We further hypothesized that COVID-19-induced coagulopathy could be a consequence of endothelial injury, based on the rationale that SARS-CoV-2 has an endothelial tropism linked to ACE2 expression. Moreover, recently an endotheliitis has been described in SARS-CoV-2 infection and could be at the origin of impaired

| TABLE 2  | Demographic, clinical, and treatment characteristics of patients on admission according to COVID-19 viral status |
|-----------|---------------------------------------------------------------------------------------------------------------|
|           | COVID-19 negative | COVID-19 positive | P-value |
| Male sex, n (%) | n = 30 | n = 66 | .05 |
| Age, years, median [IQR] | 63.0 [55.3, 75.8] | 66.0 [54.3, 79.8] | .690 |
| BMI, kg/m², median [IQR] | 24.8 [22.6, 26.3] | 26.5 [24.7, 29.1] | .110 |
| Time from illness onset to hospital admission, days, median [IQR] | 4.47 (4.57) | 5.45 (3.71) | .260 |
| CV risk factors, n (%) |                                      |                          |       |
| Hypertension | 16 (53.3) | 31 (47.0) | .720 |
| Dyslipidemia | 6 (20.0) | 21 (31.8) | .340 |
| Diabetes | 4 (13.3) | 12 (18.2) | .760 |
| Sedentarity | 6 (20.0) | 6 (9.1) | .260 |
| Chronic kidney disease | 4 (13.3) | 8 (12.1) | 1.000 |
| Medical history, n (%) |                                      |                          |       |
| Cancer | 6 (20.0) | 6 (9.1) | .240 |
| Coronary heart disease | 3 (10.0) | 7 (10.6) | .920 |
| Stroke | 0 (0.0) | 4 (6.1) | .400 |
| treatments, n (%) |                                      |                          |       |
| Statins | 3 (10.0) | 13 (19.7) | .220 |
| Oral antidiabetic agents | 2 (6.7) | 9 (13.6) | .510 |
| Insulin | 2 (6.7) | 5 (7.6) | 1.000 |
| \(\beta\)-blockers | 5 (16.7) | 8 (12.1) | .770 |
| Calcium channel blockers | 7 (23.3) | 13 (19.7) | .890 |
| ACEi or ARBs | 9 (30.0) | 21 (31.8) | 1.000 |
| Diuretics | 3 (10.0) | 6 (9.1) | 1.000 |
| Central acting agent | 1 (3.3) | 0 (0.0) | .680 |
| Curative anticoagulation | 3 (10.0) | 12 (18.2) | .470 |
| Clinical features, n (%) |                                      |                          |       |
| Fever | 22 (73.3) | 61 (92.4) | .020 |
| Headache | 1 (3.3) | 12 (18.2) | .090 |
| Cough | 14 (46.7) | 47 (71.2) | .030 |
| Productive cough | 3 (10.0) | 8 (12.1) | 1.000 |
| Dyspnea | 9 (30.0) | 31 (47.0) | .210 |
| Myalgia | 5 (16.7) | 21 (31.8) | .190 |
| Diarrhea | 2 (6.7) | 7 (10.6) | .640 |
| Pneumonia at CT scan | 11 (36.7) | 48 (72.7) | .002 |
| ARDS | 1 (3.3) | 9 (13.6) | .240 |
| SpO2 %, median [IQR] | 96.0 [92.0, 98.0] | 95.0 [91.0, 96.0] | .050 |
| Respiratory rate, breaths per minute, median [IQR] | 20.0 [16.5, 25.0] | 19.0 [16.0, 22.8] | .550 |
| Pulse, beats per min, median [IQR] | 87.0 [74.0, 100.0] | 87.0 [74.5, 103.5] | .850 |

Abbreviations:: ACEi, angiotensin conversion enzyme inhibitor; ARBs, angiotensin 2 receptor blocker; ARDS, acute respiratory distress syndrome; BMI, body mass index; CV, cardiovascular; IQR, interquartile range; SpO2, oxygen saturation.
microcirculatory function affecting particularly the lungs and kidneys. Thus, we explored CECs as a recognized non-invasive marker for endothelial lesion, as demonstrated in acute cardiovascular conditions such as acute coronary syndrome and pulmonary arterial hypertension. In agreement with the consensus protocol from ISTH, we used the reference method for CEC quantification and the threshold of 10 CECs per mL as upper normal value. We found that more than 60% of COVID-19-positive patients were above this threshold. Interestingly, patients enrolled while they were treated with curative anticoagulation had a significantly lower level of CECs, especially in the hypertensive population treated with ACEi or ARBs.

Increased mortality and/or morbidity of COVID-19 in patients with hypertension has been described in China. One of the most important concerns is the association between hypertension and treatment with ACEi or ARBs. Indeed, because ACE2 is a receptor for viral entry of SARS-CoV-2, a link between ACEi or ARBs was considered. ACEi or ARBs were described to increase ACE2 expression in the heart, brain, and even in urine after treatment. However, the main scientific societies of cardiology and more specifically of hypertension took the position not to withdraw ACEi or ARBs. Indeed, because ACE2 is a receptor for viral entry of SARS-CoV-2, a link between ACEi or ARBs was considered. ACEi or ARBs were described to increase ACE2 expression in the heart, brain, and even in urine after treatment. However, the main scientific societies of cardiology and more specifically of hypertension took the position not to withdraw ACEi or ARBs. Indeed, because ACE2 is a receptor for viral entry of SARS-CoV-2, a link between ACEi or ARBs was considered. ACEi or ARBs were described to increase ACE2 expression in the heart, brain, and even in urine after treatment.

### Table 3: Biological parameters of patients on admission according to COVID-19 viral status

| Parameter                              | COVID-19 negative | COVID-19 positive | P-value |
|----------------------------------------|-------------------|-------------------|---------|
| White blood cells, ×10^9 per L, median [IQR] | 7.8 [6.1, 11.4]   | 6.0 [4.6, 7.4]    | .008    |
| Hemoglobin, g/L, median [IQR]          | 126.0 [110.3, 141.0] | 130.5 [112.0, 144.0] | .490    |
| Platelet count, ×10^9 per L, median [IQR] | 217.5 [157.0, 278.3] | 167.5 [146.3, 223.0] | .090    |
| Neutrophils, ×10^9 per L, median [IQR] | 5.7 [4.2, 9.0]    | 4.0 [3.0, 5.9]    | .020    |
| Lymphocytes, ×10^9 per L, median [IQR] | 1.0 [0.8, 1.7]    | 0.9 [0.7, 1.3]    | .170    |
| Monocytes, ×10^9 per L, median [IQR]   | 0.6 [0.4, 0.8]    | 0.4 [0.3, 0.6]    | .300    |
| CRP, mg/L, median [IQR]                | 55.6 [3.3, 127.2] | 74.0 [22.7, 126.3] | .210    |
| Plasma creatinine level, µmol/L, median [IQR] | 99.0 [55.0, 112.0] | 79.0 [62.7, 108.7] | .970    |
| Hs-TnI, pg/mL, median [IQR]            | 10.5 [3.5, 32.2]  | 9.5 [5.1, 22.9]   | .740    |
| PT ratio, median [IQR]                 | 0.94 [0.70, 1.10] | 0.95 [0.86, 1.00] | .320    |
| Fibrinogen, g/L, median [IQR]          | 5.1 [4.3, 5.8]    | 5.3 [4.7, 6.2]    | .420    |
| D-dimers > 500 ng/mL, n (%)            | 13 [43.3]         | 49 [74.2]         | .007    |
| Fibrin monomers, µg/mL, median [IQR]   | 7.0 [7.0, 7.0]    | 7.0 [7.0, 7.0]    | .550    |
| Antithrombin, %, median [IQR]          | 99.0 [85.3, 102.8] | 101.0 [86.5, 106.5] | .500 |
| CECs per mL, median [IQR]              | 9 [6, 18]         | 19 [10, 39]       | .008    |
| CECs ≥ 10 per mL, n (%)                | 8 (27)            | 42 (64)           | .012    |

Abbreviations: CECs, circulating endothelial cells; CRP, C-reactive protein; Hs-TnI, high-sensitive troponin I; IQR, interquartile range; PT, thromboplastin time.
In our population patients treated with curative anticoagulation had a lower level of CECs, so we hypothesized that curative anticoagulation in COVID-19 patients could decrease thrombotic risk and subsequent mortality. To our knowledge, this is the first time that a potential protective effect of anticoagulant therapy on endothelial dysfunction is described (Figure 3). Besides the coagulopathy associated to endothelial lesion, the effect of anticoagulation may act directly on SARS-CoV-2 entrance in endothelial cells. Indeed, cell entry of SARS-CoV-2 depends on the binding of the viral spike (S) proteins to cellular receptors and on S protein priming by host cell proteases. It has been demonstrated that serine protease TMPRSS2 is necessary for S protein priming.35 Thus, a TMPRSS2 inhibitor has been proposed as a treatment option. If the virus’s entrance into cells is dependent on a serine protease action, anticoagulation by inhibiting thrombin activity and serine proteases of the coagulation cascade could directly limit virus entrance in endothelial but also in other cells. This hypothesis needs to be tested in preclinical models of infection.

4.1 Study limitations

Our study has several limitations. First, we are aware that false COVID-19-negative patients may exist in our study population due to the imperfect sensitivity of the diagnostic test currently used.36 Second, the study population size was necessarily small given the emergency of understanding COVID-19; indeed we decided to investigate the endothelial dysfunction associated to COVID-19 in order to consider new therapeutic alternatives. In addition, due to this small population, our results concerning anticoagulant treatment and ACEi or ARBs therapy generated hypotheses that need to be validated in larger cohorts.

In conclusion, it therefore seems consistent to open the way to curative anticoagulant treatment as part of the management of COVID-19 patients in order to limit associated endothelial dysfunction (Figure 3). Anticoagulation may not only modify COVID-19-associated coagulopathy but also pathophysiology of SARS-CoV-2 systemic dissemination. Curative anticoagulation could decrease mortality observed in COVID-19 patients. Further studies should evaluate safety and efficacy of curative anticoagulation in COVID-19 patients to prevent worsening of disease and reduction of admittance in ICUs.

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FIGURE 2 Effect of curative anticoagulation on circulating endothelial cell (CEC) levels in COVID-19. Quantification of CECs in COVID-19-positive patients at admission. CEC level according to presence or the absence of curative anticoagulation and/or the presence or the absence of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs). Red dotted line shows the upper limit of reference values for CECs (<10 CECs per mL)

FIGURE 3 Curative anticoagulant treatment could be part of COVID-19 management in order to limit associated endothelial dysfunction

with anticoagulation

No anticoagulation

ACE2

SARS-CoV-2

CECs

With anticoagulation

No anticoagulation
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AUTHOR CONTRIBUTIONS
D. M. Smadja, T. Mirault, and J.-L. Diehl interpreted data, and conceived and supervised the study. L. Khider and N. Gendron interpreted data and drafted the manuscript. R. Chocron analyzed the data and supervised statistical analysis. L. Khider, N. Gendron, G. Goudot, R. Chocron, and B. Debuс analyzed the data and reviewed all patients’ characteristics. All authors interpreted data, drafted and revised the manuscript, and approved the final version.

CONFLICTS OF INTEREST
All the authors have nothing to disclose.

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