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Letter to the Editors-in-Chief

Are antiphospholipid antibodies associated with thrombotic complications in critically ill COVID-19 patients?

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1. Introduction

Coronavirus disease 2019 (COVID-19) patients are at high risk of thrombosis related to endothelium injury, low blood flow and marked hypercoagulability [1]. Recently, a high prevalence of lupus anticoagulant (LA) was reported in the COVID-19 patients [2], immediately questioned by the possibility of false positive testing given the marked elevation in C-reactive protein (CRP) levels attributed to the major pulmonary or systemic inflammation in these patients [3]. Interestingly, a strong association between thrombosis and the presence of LA in critically ill COVID-19 was suggested but not demonstrated [4]. Moreover, the contribution of anticardiolipin and anti-β2-glycoprotein-I antibodies to COVID-19-associated thrombosis was suggested in three patients with multiple cerebral infarctions, although no information on their detection and IgA/IgG titers was given [5]. Altogether, only few data were provided regarding anticardiolipin and anti-β2-glycoprotein-I antibodies [2,4].

Therefore, we aimed to investigate the prevalence of elevated antiphospholipid antibodies, namely LA, anticardiolipin IgG/IgM and anti-β2-glycoprotein-I IgG, and their possible association with thrombotic complications in COVID-19 patients.

2. Subjects and methods

We conducted a prospective single-center observational study including all consecutive critically ill COVID-19 adults admitted from March 23 to April 15, 2020. SARS-CoV-2 infection was diagnosed using standard RT-PCR technique (Cobas-SARS-CoV-2 kits®, Roche, France). This study was part of the ICU-COVID cohort registry approved by our institutional ethics committee. Duplex ultrasound was systematically performed once weekly to diagnose proximal and distal lower extremity deep vein thrombosis. If suspected, pulmonary embolism was confirmed using computed-tomography/angiography. Patients were treated with prophylactic enoxaparin or unfractionated heparin (UFH) on admission, switched to a therapeutic dose regimen in case of extracorporeal membrane oxygenation or thromboembolic event diagnosis. All laboratory results were obtained within 24 h of the ultrasound assessment. β_2-Glycoprotein-I-dependent anticardiolipin IgG/IgM and anti-β_2-glycoprotein-I IgG were quantified using chemiluminescence assays (Acustar®, Werfen). LA diagnosis was made using integrated diluted Russell viper venom time (dRVVT LAC-Screen/Confirm®, Siemens) and LA-sensitive activated partial thromboplastin time (aPTT) (PTT-LA® for screen, then Staclot-LA®, Stago), including mixing studies for both dRVVT and aPTT-based tests using Pool-Norm-Plasma® (Stago) if needed. Patient dRVVT screen and confirm results were expressed as ratios versus reference plasma results. Cut-off value was 1.20 for both screen ratio and, if positive, screen ratio/confirm ratio. Data are presented as median [25th–75th percentile] or numbers (percentages) as appropriate. Comparisons of the patient characteristics according to the presence of thrombotic complications were performed using Mann-Whitney or Fisher's exact tests as required. Differences with $p < .05$ were considered significant.

3. Results

Seventy-four consecutive mechanically ventilated patients were included. On admission, they received prophylactic (73%) or therapeutic (27%) enoxaparin or UFH. None had received any other anticoagulant drug before ICU admission. Thrombotic events were reported in 28 patients (38%) and included 26 deep vein thrombosis, 4 pulmonary embolisms, 1 stroke and 1 extensive venous catheter thrombosis. Patients with thrombosis exhibited significantly higher plasma D-dimer ($p = .0003$), serum creatinine ($p = .02$) and serum lactate dehydrogenase ($p = .03$), as well as a trend to more marked hypoxemia ($p = .08$; Table 1).

Overall, antiphospholipid antibodies, namely LA and/or elevated anticardiolipin IgG/IgM and/or elevated anti-β_2-glycoprotein-I IgG, were present in 88% of the patients. LA, based on dRVVT system, was positive in 63 patients (85%) but not associated with thrombotic complications ($p = .7$; Fig. 1). Noteworthy, our dRVVT results could be interpreted since UFH or enoxaparin anti-Xa activity was systematically measured and found < 0.9 IU/mL in all samples, thus excluding heparin interference with dRVVT results, due to the presence in the reagent of an heparin quenching agent effective until 1.0 IU/mL as specified by the manufacturer and checked locally for accreditation [6,7]. Moreover, anti-Xa activity results did not differ significantly between

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Table 1
Data in seventy-four critically ill COVID-19 patients in relation to the development of thrombotic complications.

	Patients with thrombotic complications (N = 28)	Patients without thrombotic complications (N = 46)	*P* value
Demographics and past medical history			
Age (years)	63 [57–69]	64 [51–72]	1.0
BMI (kg/m^2)	29.4 [25.1–31.7]	28.0 [24.7–31.1]	0.5
Diabetes, N (%)	12 (43)	18 (39)	0.9
Hypertension, N (%)	12 (43)	21 (46)	1.0
Ischemic heart disease, N (%)	4 (14)	10 (22)	0.5
Autoimmune disease, N (%)	3 (11)	7 (15)	0.7
Conditions of measurement			
Time from COVID-19 symptoms to measurement (days)	10 [7–12]	10 [9–13]	0.3
Time from COVID-19 diagnosis to measurement (days)	5 [1–8]	4 [3–8]	0.1
Coagulation tests			
Prothrombin time (ratio)	0.78 [0.67–0.84]	0.81 [0.73–0.91]	0.2
Fibrinogen (g/L)	7.6 [6.2–8.8]	8.0 [6.4–8.8]	0.8
D-dimer (ng/mL)	5590 [2793-7728]	2120 [1050–3300]	0.0003
Positive dRVVT*, N (%)	23 (82)	40 (87)	0.7
Elevated anticardiolipin IgG/IgM and/or anti-β2-glycoprotein-I IgG antibodies**, N (%)	5 (18)	4 (9)	0.3
Other laboratory parameters			
PaO_2/FiO_2 (mmHg)	173 [90–236]	111 [84–165]	0.08
Blood lymphocytes (G/L)	0.9 [0.6–1.2]	0.9 [0.5–1.1]	0.7
Platelets (G/L)	293 [191–398]	263 [191–360]	0.9
Serum creatinine (μmol/L)	98 [79–220]	75 [61–122]	0.02
Serum alanine aminotransferase (IU/L)	37 [28–50]	37 [27–62]	0.9
Serum lactate dehydrogenase (IU/L)	645 [471–852]	503 [434–633]	0.03
C reactive protein (mg/L)	203 [75–267]	158 [66–258]	1.0
Procalcitonin (μg/L)	0.68 [0.24–1.13]	0.46 [0.23–1.25]	0.7
Interleukin-6 (pg/mL)	82 [12–163]	70 [20–161]	0.7
Viral load (cycles) (Cobas SARS-CoV-2 kits®)	30.1 [25.3–31.0]	26.5 [23.0–30.5]	0.2
Treatment			
Hydroxychloroquine, N (%)	5 (18)	16 (34)	0.9
Azithromycin, N (%)	8 (29)	18 (39)	0.7
Lopinavir/ritonavir, N (%)	1 (4)	12 (26)	0.9
Dexamethasone, N (%)	4 (14)	13 (28)	0.8
Renal replacement therapy, N (%)	9 (32)	10 (22)	0.7
Outcome			
Outcome (death/in ICU/discharged), N (%)	7 (25)/5 (18)/16 (57)	12 (26)/4 (8)/30 (65)	0.1

Data are presented as median [25th–75th percentile] or numbers (percentages) as appropriate.
BMI, body mass index; COVID-19, Coronavirus disease 2019; ICU, intensive care unit; *patient dRVVT screen (low phospholipid concentration) and confirm (high phospholipid concentration) results were normalized, i.e. expressed as ratios versus reference plasma results. Results are expressed as screen ratio/confirm ratio. Cut-off value was 1.20 for both screen ratio and screen ratio/confirm ratio, demonstrating the phospholipo-dependence; **anticardiolipin IgG/IgM and/or anti-β2-glycoprotein-I IgG antibodies was defined as elevated if the titer was > 20 CU (99th percentile).

patients with positive and negative LA (0.20 IU/mL [0.13–0.62] versus 0.25 IU/mL [0.10–0.36], $p = .3$). Otherwise, elevated CRP levels did not interfere with the integrated dRVVT test system. By contrast, aPTT-based LA results could not be interpreted as i/- PTT-LA® assay is affected by both UFH/enoxaparin anti-Xa activity despite sampling preferably performed just before injection; ii/- both PTT-LA® and Staclot-LA® are affected by elevated CRP levels so that false positive results could not be excluded in these COVID-19 patients [3,6–8]. Finally, we could evidence that LA could be transient in a subset of patients during the ICU stay: when reassessed once nine days later on average in the 31 patients who were still hospitalized, LA appeared labile, mainly positive turning to negative (N = 9), versus negative to positive (N = 3).

Nine patients (12%) had elevated anticardiolipin IgG/IgM and/or anti-β_2-glycoprotein-I IgG [titer ranges, 23–100 CU (N = 7), 24–237 (N = 2) and 21–64 (N = 3)], including seven with positive LA and two with negative LA. These patients presented no significantly different characteristics but tended to have been tested longer after COVID-19 symptoms started than the negative patients ($p = .06$). Interestingly, one patient with isolated positive anticardiolipin IgM initially switched thereafter to positive anticardiolipin IgG/IgM and anti-β_2-glycoprotein-I IgG. Patients with positive anticardiolipin/anti-β2-glycoprotein-I antibodies had no significantly increased thrombosis risk during ICU stay ($p = .3$). Remarkably, the only patient with the triple positive antiphospholipid antibodies (anticardiolipin IgG, 100 CU and IgM, 2 CU; anti-β_2-glycoprotein-I IgG, 64 CU; positive dRVVT) died from massive pulmonary embolism.

4. Discussion

Compared to other viral and bacterial infections known to trigger transient antiphospholipid antibodies [9], LA prevalence was extremely high (85%) in critically ill COVID-19 patients, similar to Helms' study (87.7%) also conducted in ICU patients [4]. This elevated prevalence could be attributed to the cytokine storm-related inflammation and dysimmunity. By contrast to Helms et al. [4], we did not find any significant association between LA and thrombosis, consistent with reports about other viral infections [8]. Moreover, the lability of LA within a short time that we showed in a subset of patients requires further investigation. The concomitant use of immunomodulatory agents such as hydroxychloroquine known to potentially negative the presence of LA [10], makes the interpretation complex. At least, the presence of LA should require the attention of physicians in charge of COVID-19 patients with thrombotic complications as it may prolong the activated partial thromboplastin time, hampering UFH monitoring and making anti-Xa activity measurement mandatory in this setting.

We found a moderately elevated prevalence of anticardiolipin/anti-β_2-glycoprotein-I antibodies (12%) in our COVID-19 patients, in the same order of magnitude as Harzallah'study prevalence (10%) [2]. One suggested mechanism is cross-reacting antibodies involving antigenic similarities between viral antigens and the host β_2-glycoprotein-I, used as molecular mimicry [9]. In critically ill COVID-19 patients, cross-reactivity may be facilitated by β_2-glycoprotein-I exposure subsequent to major endothelial injury. However, whether these antibodies could be responsible for increased thrombotic risk remains uncertain, depending on their titer, isotype and persistence. This association is clearly questionable in the three patients described by Zhang et al. [5]. In COVID-19 patients with thrombosis, persistence period of antiphospholipid antibodies over a 3-month period is required before considering the diagnosis of antiphospholipid syndrome.

Limitations of the current study include the relatively small number of patients, the single-center setting, and the short study period. However, to the best of our knowledge, this is the first study evaluating the prevalence of both LA and anticardiolipin/anti-β2-glycoprotein-I antibodies in COVID-19 patients. Because we focused on critically ill COVID-19 patients, prevalence of antiphospholipid antibodies might be different in less severe patients.

In conclusion, we confirm that LA are highly prevalent but conclude that despite its high prevalence, LA are not associated with thrombosis occurrence reported in the COVID-19 patients. Whether anticardiolipin/anti-β2-glycoprotein-I antibodies play a role in the pathophysiology of COVID-19-attributed thrombosis remains to be clarified in larger series. Finally, whether antiphospholipid antibodies are

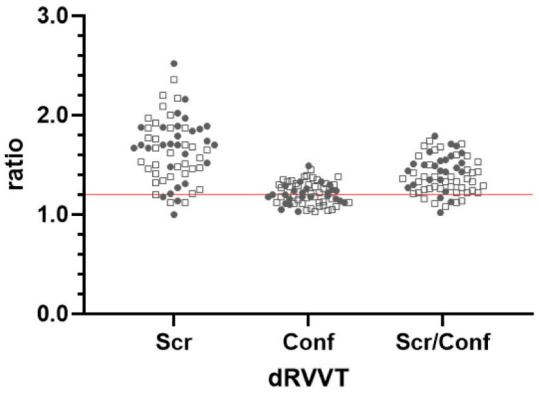

Fig. 1. Lupus anticoagulant (LA) results in COVID-19 patients with (black circles) and without thrombotic events (open squares). Patient dRVVT screen (Scr, low phospholipid concentration) and confirm (Conf, high phospholipid concentration) results were normalized, i.e. expressed as ratios against reference plasma results. Final results were expressed as screen ratio/confirm ratio. Cut-off value was 1.20 for both screen ratio and screen ratio/confirm ratio, demonstrating the phospholipo-dependence.

transient or persistent needs to be determined.

Author contributions

VS and BM designed the study. SV, EG and BM took care of the patients VS and AS performed the biological tests. All authors acquired, analyzed and interpreted the data. VS, AS and BM drafted the first version of the manuscript. All authors critically revised the manuscript and participated in final approval for publication. BM had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration of competing interest

None.

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