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Antiphospholipid antibodies and thrombotic events in COVID-19 patients hospitalized in medicine ward

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Dear Editor,

Rapidly after the first description of the new coronavirus disease (COVID-19) [1], thrombotic events were increasingly reported as a common complication [2–7]. Autopsy series further revealed deep venous thrombosis in up to 58% of severe COVID-19 patients in whom venous thromboembolism was not suspected before death [8]. In scarce studies reporting thromboembolic events in COVID-19 patients hospitalized in a medicine ward, the prevalence of thrombotic complications appeared lower, from 6.6 to 14% [6,7,9,10]. Underlying mechanisms that may increase the risk of thrombotic events in COVID-19, called "COVID-19 coagulopathy", remain poorly understood. A role for antiphospholipid antibodies (aPL) has been suggested [10,11]. In the present prospective single-center observational cohort study of COVID-19 patients admitted in the Internal Medicine Department of a tertiary care university hospital (Paris, France), we aimed to describe the prevalence of antiphospholipid markers in a consecutive series of non-ICU COVID-19-patients and to further assess whether there is a relationship between the presence of aPL and the occurrence of thrombotic events.

Included patients were older than 18 years with an initial requirement for hospitalization in a medical ward and a positive SARS-CoV-2 RT-PCR assay from nasal swabs. Patients were tested for the presence of aPL antibodies, i.e., aCL, aβ2GPI and for a subgroup, lupus anticoagulant. The ELISA QUANTALite™ (Inova Diagnostics, San Diego, CA) and the EliA β-2 Glycoprotein-1 (Phadia, Uppsala, Sweden) detection kits were used to detect IgG/IgM/IgA aCL and IgG/IgM/IgA aβ2GPI antibodies, respectively, with a limit of positivity fixed at 15 units/mL (99th percentile of a control population). Lupus anticoagulant (LA) testing was performed on a CS5100 analyzer according to the International Society on Thrombosis and Haemostasis guidelines. Thrombotic event was defined as a venous thrombosis or an arterial thrombotic event on admission or during follow up. Patients were followed-up until discharge, death, or up to 14 days. All patients benefited from the current standard of care for COVID-19 and received thromboprophylaxis according to current guidelines. Continuous variables are presented as median (interquartile range, IQR) and were compared using Wilcoxon's-test. Categorical variables are presented as count (percent) and were compared using Fisher's-test. All statistics were two-sided at a 5% significance level. Analyses were computed with IBM SPSS Statistics software (IBM Corp, Armonk, USA). The study followed the Strengthening Reporting of Observational Studies in Epidemiology for cohort studies. We received approval from the local ethiccommittee, and our study is registered as NCT04320017.

Between March 20 and April 23, 2020, a total of 104 patients were included. Main baseline features are presented in Table 1. The median age was 71 years [59–81] and 60 (57.7%) patients were males. Patients had a past medical history of venous thrombosis, stroke and peripheral arterial disease in 15.4%, 10.6% and 8.7%, respectively. C-reactive protein (CRP) and D-dimer levels were both increased with median values of 69 mg/L [30–107] and 950 mg/L [480–1920], respectively. These general characteristics are in accordance with previously published series of COVID-19 patients [1].

Eleven (10.6%) patients presented a thrombotic event on admission or during follow up, i.e., 9 acute pulmonary embolisms, 1 deep vein thrombosis and 1 aortic thrombus. The main differences in patient characteristics according to the presence of a thrombotic event are detailed in Table 1. Patients with a thrombotic event had more frequently a past medical history of venous thrombosis (36.4% vs. 13.9%) and higher levels of neutrophil count (6460 vs. 4420/mm^3, $p = 0.019$), CRP (124 vs. 64.2 mg/L, $p = 0.021$), and D-Dimer (5860 vs. 890 µg/L, $p < 0.001$). Most previous studies evaluating the prevalence of thrombotic events in COVID-19 have been conducted in critically ill patients. Helms et al. reported up to 43% of clinically relevant thrombotic events[3]. In a series of 184 critically ill COVID-19 patients, pulmonary embolism and deep vein thrombosis were found in 13.6% and 0.5%, respectively [2]. Venous thrombotic events have been associated with higher D-Dimer levels and prolonged aPPT [4]. Other studies reported pulmonary embolism in 6.6 to 10% in non-ICU patients [6,7,9,10].

In the present study, the presence of aCL was noted in 35/104 (33.7%) patients, mostly IgA aCL. Anti-β2-GPI were found in 9/104 (8.7%) patients. IgG, IgM and IgA aβ2-GPI were positive in 8.7%, 2.9% and 5.8%, respectively. Lupus anticoagulant was found positive in 21 out of 53 (39.6%) patients. Overall, 49/104 (47.1%) patients had a least

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Table 1
Patients characteristics and antiphospholipid markers in a prospective cohort of COVID-19 patients.

	All patients	Patients without thrombotic event	Patients with thrombotic event	*P* value
Number of patients	*N* = 104	*N* = 93	*N* = 11	
Demographics				
Age, median [IQR] years	71 [59–81]	71 [59–80.5]	80 [60–84]	0.328
Male, no. (%)	60 (57.7)	53 (57)	7 (63.6)	0.756
Smoking, n (%)	10 (9.8)	10 (10.9)	0 (0)	0.592
BMI (kg/m^2)	24.4 [22.5–28.6]	25.7 [23.1–29.6]	24.2 [21.26.3]	0.359
Past medical history, n (%)				
Venous thrombosis	16 (15.4)	12 (13.9)	4(36.4)	0.064
Cancer	27 (26.2)	25 (27.2)	2 (18.2)	0.753
Active cancer	18 (17.3)	16 (17.2)	2 (18.2)	1
Coronary ischemic disease	18 (17.3)	16 (17.2)	2 (18.2)	1
Stroke	11 (10.6)	10 (10.8)	1 (9.1)	1
Peripheral arterial disease	9 (8.7)	8 (8.6)	1 (9.1)	1
Hypertension	61 (58.7)	55 (59.1)	6 (54.5)	0.759
Dyslipidemia	39 (37.5)	32 (34.4)	7 (63.6)	0.096
Diabetes	25 (24)	21 (22.6)	4 (36.4)	0.454
Platelet inhibitor	24 (23.1)	20 (21.5)	4 (36.4)	0.273
Oral anticoagulant	15 (14.4)	14 (15.1)	1 (9.1)	0.506
Covid-19 symptoms, n (%)				
Fatigue	65 (62.5)	60 (64.5)	5 (45.5)	0.393
Dyspnea	59 (56.7)	51 (54.8)	8 (72.7)	0.342
Cough	45 (43.3)	39 (41.9)	6 (54.5)	0.577
Diarrhea	21 (20.2)	20 (21.5)	1 (9.1)	0.301
Dysgueusia	20 (19.2)	18 (19.4)	2 (18.2)	1.0
Thoracic pain	19 (18.3)	15 (16.1)	4 (36.4)	0.313
Anosmia	12 (11.5)	11 (11.8)	1 (9.1)	1.0
Hemoptysis	2 (1.9)	0 (0)	2 (18.2)	0.016
Covid-19 course				
Nasal oxygen (L/min)	2 [2–4]	2 [2–3]	4 [3–11.5]	0.01
ICU transfer D14, n (%)	17 (16.3)	14 (15)	3 (27)	0.437
Mortality Day 14, n (%)	14 (13.5)	11 (11.8)	3 (27.3)	0.16
Baseline laboratory findings				
Neutrophils, /mm^3	4535 [3185–6047]	4420 [2930–5615]	6460 [4537–8755]	0.019
Lymphocytes, /mm^3	955 [710–1237]	950 [710–1210]	1020 [717–1480]	0.600
Haemoglobin, g/dL	12.1 [11.1–13.9]	12.3 [11.1–13.9]	12 [11.2–13.0]	0.534
Platelet count, G/mm^3	220 [159–288]	220 [159–282]	231 [153–368]	0.355
CRP, mg/L	69 [30–107]	64.2 [28.3–104.1]	124 [64.7–253]	0.021
Fibrinogen, g/L	6.0 [4.83–6.98]	5.95 [4.88–6.93]	6.5 [4.5–8.15]	0.861
D-Dimer, µg/L	950 [480–1920]	890 [450–1615]	5860 [2555–17,750]	<0.001
Ferritin, µg/L	876 [364–1463]	867 [356–1526]	979 [368–1413]	1.0
IL-6, pg/mL	60 [34–83]	61.5 [34.8–91.8]	54 [31.8–123]	0.980
Anti-phospholipid Ab markers				
IgG anticardiolipin Ab	8 (7.7)	5 (5.4)	3 (27.3)	0.037
IgG titer (fold UNL)	1.5 [1.3–2]	1.6 [1.2–2]	1.3/1.5/2.3*	0.786
IgM anticardiolipin Ab	8 (7.7)	3 (3.2)	5 (45.5)	< 0.001
IgM titer(fold UNL)	1.6 [1.4–2.2]	1.2/1.5/1.6*	1.7 [1.5–2.5]	0.143
IgA anticardiolipin Ab	31 (28)	26 (28)	5 (45.5)	0.297
IgA titer (fold UNL)	1.5 [1.4–1.7]	1.4 [1.3–1.7]	1.7 [1.6–2.6]	0.081
IgG anti-β2-GPI Ab	5 (4.8)	4 (4.3)	1 (9.1)	0.435
IgG titer (fold UNL)	4.5 [1.8–14]	6.9 [1.6–15.1]	4.5*	–
IgM anti-β2-GPI Ab	3 (2.9)	2 (2.2)	1 (9.1)	0.287
IgM titer(fold UNL)	1/4/26*	4/26*	1*	–
IgA anti-β2-GPI Ab	6 (5.8)	3 (3.2)	3 (27.3)	0.015
IgA titer (fold UNL)	10 [3.1–13]	2.6/3.3/15.2	10/10/12	0.7
Lupus anticoagulant, yes/nb tested	21/53 (39.6)	18/48 (37.5)	3/5 (60)	0.374
Anti-phospholipid markers positivity#				
Single positivity	35 (33.7)	31 (33.3)	4 (36.4)	0.740
Double positivity	12 (11.5)	11 (11.8)	1 (9.1)	1
Triple positivity	2 (1.9)	0 (0)	2 (18.2)	0.01

IQR: InterQuartil Range; BMI: Body Mass Index; CRP: C reactive protein; Ab: antibody.

Positivity of anti-phospholipid marker among anticardiolipin Ab, anti-β2-GPI Ab and lupus anticoagulant.

* When n ≤ 3, singles values are given.

one positive aPL marker while double or triple antiphospholipid seropositivity was found in 11.1% and 1.9%, respectively. Then, we analyzed the results of aPL positivity according to the presence of thrombotic events. Anticardiolipin antibodies were more frequently found in patients with thrombotic events. The only aCL isotypes significantly associated with thrombotic events were IgG and IgM aCL ($p = 0.037$ and $p < 0.001$, respectively). IgA aβ2-GPI were more frequently found in patients with thrombotic events [3/11 (27.3%) vs. 3/93 (3.2%), $p = 0.015$)]. Lupus anticoagulant was found to be positive in 60% (3/5) of patients with thrombotic event vs. 37.5% (18/48, $p = 0.374$) of those without. Triple aPL positivity was seen in 2/11 (18.2%) versus none (0/93) of the patients with and without thrombotic events ($p = 0.01$). In the first patient, a 80-year-old man with a thrombus of the ascendant aorta, LA, aCL (IgG and IgA), and aβ2-GPI (IgG, IgM, IgA) were found positive. The other patient was an 83-year-old man with a history of multiple deep venous thromboses and a factor V deficiency who had a recent deep venous thrombosis. Lupus anticoagulant, IgM and IgA aCL and IgA aβ2-GPI were positive. If we consider only highly positive aPL (cut off >40 U/mL), 26/103 (25.2%) non-ICU patients had at least one positive aPL [4/11 (36%) vs 22/91 (24%) patients with or without thrombosis, $p = 0.461$]. The presence of at least two positive

aPL (>40 U/ml) was associated with thrombosis [3/11 (27.3%) vs 1/93 (1.1%), $p = 0.004$]. Overall, almost half of non-ICU COVID-19 patients had at least one aPL, mainly aCL whereas aβ2-GPI were more rarely found (<10%). Viral-induced aCL are rarely associated with aβ2-GPI, often transient and are not correlated with thrombosis risk [12]. One limitation of this study is that we could not assess if these aPL were persistent are not. In a COVID-19 case series of 216 patients, lupus anticoagulant was found in 31 out of 35 of those with a prolonged aPPT, from which only two had suspected or confirmed venous thrombotic events [13]. Moreover, a series of 150 critically ill patients showed lupus anticoagulant in 50 out of 57 patients tested [3].

In conclusion, antiphospholipid antibodies, even weak and/or transient, are common in COVID-19 patients hospitalized in a medicine ward. In this prospective cohort, 64% of patients with a thrombotic event were found to have aPL antibodies. Only the presence of aCL and aβ2-GPI antibodies were significantly associated with the occurrence of thrombotic events. Although our results suggest that antiphospholipid antibodies are common in non severe COVID-19, their relationship with thrombosis and COVID-19 associated coagulopathy will necessitate more dedicated studies.

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Disclosure of conflicts of interest

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