Received: 18 May 2020 | Accepted: 1 July 2020

DOI: 10.1111/jth.14994

ORIGINAL ARTICLE

Antiphospholipid antibodies in patients with COVID-19: A relevant observation?

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Abstract

Background: High incidence of thrombosis in COVID-19 patients indicates a hypercoagulable state. Hence, exploring the involvement of antiphospholipid antibodies (aPL) in these patients is of interest.

Objectives: To illustrate the incidence of criteria (lupus anticoagulant [LAC], anticardiolipin [aCL] immunoglobulin G [IgG]/IgM, antibeta2-glycoprotein I antibodies [aβ2GPI] IgG/IgM) and noncriteria (anti-phosphatidyl serine/prothrombin [aPS/PT], aCL, and aβ2GPI IgA) aPL in a consecutive cohort of critically ill SARS-CoV-2 patients, their association with thrombosis, antibody profile and titers of aPL.

Patients/Methods: Thirty-one consecutive confirmed COVID-19 patients admitted to the intensive care unit were included. aPL were measured at one time point, with part of the aPL-positive patients retested after 1 month.

Results: Sixteen patients were single LAC-positive, two triple-positive, one double-positive, one single aCL, and three aCL IgG and LAC positive. Seven of nine thrombotic patients had at least one aPL. Sixteen of 22 patients without thrombosis were aPL positive, amongst them two triple positives. Nine of 10 retested LAC-positive patients were negative on a second occasion, as well as the double-positive patient. Seven patients were aPS/PT-positive associated to LAC. Three patients were aCL and aβ2GPI IgA-positive.

Conclusion: Our observations support the frequent single LAC positivity during (acute phase) observed in COVID-19 infection; however, not clearly related to thrombotic complications. Triple aPL positivity and high aCL/aβ2GPI titers are rare. Repeat testing suggests aPL to be mostly transient. Further studies and international registration of aPL should improve understanding the role of aPL in thrombotic COVID-19 patients.

KEYWORDS

antibodies, antiphospholipid, antiphospholipid syndrome, COVID-19, lupus anticoagulant, thrombosis

Manuscript handled by: Robert Gosselin

Final decision: Robert Gosselin and 01-Jul-2020

1 | INTRODUCTION

Since the description of the first patients with coronavirus disease 2019 (COVID-19)-associated pneumonia, there is a growing understanding of the derangement of hemostasis in these patients.[1-3] Although the clinical evolution in coronavirus 2 (SARS-CoV-2) infected patients with severe acute respiratory syndrome is mostly favorable, patients may develop acute respiratory insufficiency requiring admittance in the intensive care unit (ICU).[4] Also, many patients develop a hypercoagulable state influencing the unfavorable clinical outcome.[3,5] Several hemostasis laboratory parameters are disturbed pointing to a coagulopathy.[2-6] Recently reports have been published on antiphospholipid antibodies (aPL) in SARS-CoV-2 patients.[5,7-9] Investigators started to measure aPL in these patients because of the hypercoagulable state.

Indeed, antiphospholipid syndrome (APS) is an important required cause of thrombotic complications, and is defined by the presence of aPL.[10] In the current classification criteria for APS lupus anticoagulant (LAC), anticardiolipin (aCL), and antibeta2-glycoprotein I antibodies (aβ2GPI) immunoglobulin G (IgG) or IgM are included as laboratory criteria, if persistently present.[10,11] APS is a challenging diagnosis as the incidence of thrombosis is high and often determined by underlying factors not related to aPL resulting in overdiagnosis.[12,13] To prevent misdiagnosis, the diagnostic workup for a patient with thrombosis requires besides adequate testing. a good collaboration between the clinician and the laboratory.[14]

The information on aPL in SARS-CoV-2 patients that is available so far is interesting, but often incomplete. Inherent to the recent development of the pandemic COVID-19 situation, in these patients only one point of measurement is obtained without confirmation after at least 3 months, as defined in the laboratory criteria of APS.[10] aPL can arise transiently in patients with critical illness and various infections.[15] The presence of these antibodies may rarely lead to thrombotic events that are difficult to differentiate from other causes of multifocal thrombosis in critically ill patients. To further investigate the role of aPL in SARS-CoV-2 patients, it is important to report all criteria aPL, including LAC, aCL, and aβ2GPI antibodies, the latter with their isotype and titer. This information is often lacking in the published reports. Measuring LAC, aCL, and aβ2GPI allows to make antibody profiles that help in identifying patients at risk.[10]

Current criteria recommend increased levels of IgG and IgM aCL and aβ2GPI to confirm APS.[10] The role of IgM aPL has been discussed based on a less strong association with thrombosis compared with IgG.[16-18] In a recent study, we illustrated that IgM was not an independent risk factor for thrombosis, but addition of IgM aCL and aβ2GPI to LAC and aCL IgG and aβ2GPI IgG increased the odd ratio for thrombosis, suggesting that testing for IgM might be useful to improve thrombotic risk stratification.[18] Previously, it was demonstrated that the presence of aCL and aβ2GPI of the same isotype reinforces the clinical probability of APS.[19] In the first report on aPL in patients with COVID-19, three patients were described with IgA (and IgG) aCL and aβ2GPI, without LAC positivity.[7] IgA aCL and aβ2GPI are not included in the current classification criteria.[10,11,20] In most cases with thrombosis, IgA aPL are usually found in association with IgG and/or IgM.[21]

The association with other aPL, such as anti-phosphatidyl serine/prothrombin (aPS/PT) merit also attention. Recent literature described their association with thrombosis.[22,23] In the published series of COVID-19 patients aPS/PT is not included.

In this report, we illustrate the presence of criteria and noncriteria aPL, including LAC, aCL (IgG, IgM, IgA), aβ2GPI (IgG, IgM, IgA), and aPS/PT (IgG and IgM), in a cohort of critically ill patients with SARS-CoV-2 and discuss the relevance.

Essentials

- COVID-19 patients develop a hypercoagulable state influencing unfavorable clinical outcome.
- Antiphospholipid antibodies (aPL) have been demonstrated in COVID-19 patients.
- Critically ill patients shows mainly single positive lupus anticoagulant, mostly transient.
- The causality between aPL and thrombosis is unclear.

2 | MATERIALS AND METHODS

2.1 | Measurement of aPL

Three-step LAC testing was carried out in a dRVVT, dilute Russell's viper venom time (dRVVT)- and activated partial thromboplastin time (APTT)-based test system according to International Society on Thrombosis and Haemostasis (ISTH) guidelines.[24] All tests were carried out on a STA-R Evolution analyzer (Stago, Asnières, France) using Stago STA-Staclot dRVV Screen, STA-Staclot DRVV Confirm, PTT-LA, and Staclot LA reagents. When dRVVT confirm results exceeded the local cutoff values, screen mix/confirm mix ratios were applied in the confirmation step.[25] Conclusions based on screening, mixing, and confirmatory steps were formulated for each test system, together with a final conclusion of positivity or negativity for LAC. This is important to check the C-reactive protein (CRP) levels to avoid false positive conclusions if only the APTT system is positive because the APTT-based test system is prone to interferences by CRP.[12,26] Applying the three-step procedure, unfractionated heparin (UFH) do not result in false-positive LAC, whereas enoxaparin cause false-positive APTT-based LAC at supra-therapeutic anti-Xa activity levels that exceed the heparin neutralizing capabilities of the reagents.[27,28] In each sample, we checked the anti-Xa level to avoid false conclusions.

aCL and aβ2GPI IgG, IgM, and IgA were measured by ACL AcuStar (Werfen/Instrumentation Laboratories). A cutoff value of 20 U/mL was applied[29-31] as previously described or transferred from the manufacturer for IgA.[20] aPS/PT IgG and IgM was measured by QUANTA Lite ELISA (Inova Diagnostics) with a cutoff value of

30 U/mL transferred from the manufacturer.[20] Solid phase assays were performed according to the ISTH guidelines.[20]

2.2 | Patient population

Thirty-one consecutive patients with confirmed COVID-19 admitted into the Ghent University Hospital ICU between March 11 and April 9, 2020, were included. The study was approved by the local ethical committee. Patient characteristics are listed in Table 1.

All patients received prophylactic or therapeutic dose low molecular weight heparin (LMWH) (enoxaparin) or UFH (Table 1). It is local practice to choose UFH under a calculated creatinine clearance of 30 mL/min. By this, when patients had deteriorating or improving kidney function during their stay at ICU, it was possible that the treating physician switched from LMWH to UFH therapy or vice versa. UFH is also chosen during extracorporeal membrane oxygenation (ECMO) therapy because dose changes are more frequent and thus the desired heparinization effect can be adjusted more quickly. Measurement of anti-Xa levels (chromogenic assays STA-Liquid anti-Xa, Stago) were performed routinely to ensure prophylactic/therapeutic levels of both LMWH and UFH heparin, taking the coagulopathy and interaction with acute phase proteins into account.[32] Dose adjustments where done based on peak anti-Xa concentration from the third dose of enoxaparin, in case of LMWH therapy. UFH therapy was titrated on steady-state levels of UFH every 6 hours. In highly inflammatory patients and in every ECMO patient, antithrombin levels were measured at the beginning of the LMWH or UFH heparin, but also if there were clinical concerns doubting appropriate levels. Patients were routinely tested for D-dimers.

Thrombotic complications were confirmed by duplex ultrasound in case of deep venous thrombosis (DVT) or central venous catheter (CVC) thrombosis, the one patient with stroke underwent computed tomography angiography. Circuit devices were assessed routinely by visual inspection for the presence of clots.

3 | RESULTS

Median age in the patient population was 63 (range, 38-82) years, with a male/female ratio of 28/3. The median stay at the ICU was 25 (range, 5-60) days. 26 patients received mechanical ventilation, five ECMO, and five renal dialysis. Anticoagulant therapy, medical history, and comorbidity, as well as thrombotic complications are shown in Table 1.

In all patients (n = 31) LAC, aCL, and aβ2GPI IgG, IgM, and IgA, aPS/PT IgG, and IgM were measured. Results are shown in Tables 2 and 3.

Eight of 31 patients were negative for all criteria aPL (LAC, aCL, and aβ2GPI IgG and IgM), 23 patients had at least one aPL positive. Twenty-one of 31 patients were LAC-positive. One (patient 15) of 21 positive LAC patients was positive only in the APTT system, but we are confident that this is not a false-positive result because CRP was elevated up to 57 mg/L and routine APTT (PTT-A, Stago) was more prolonged then expected according to the CRP level,[26,32] and

TABLE 1 Patient characteristics

	ICU Population (n = 31)	
Age (y), median (range)	63	(38-82)
Survivors, n (%)	27	(87.1)
Male/female ratio	28/3	
Medical history/comorbidity, n (%)		
Cardiovascular disease	14	(45.2)
Hypercholesterolemia	3	(9.7)
Thromboembolic event	1	(3.2)
Diabetes	8	(25.8)
Obesity	4	(12.9)
Chronic renal disease	6	(19.4)
Acute renal disease	1	(3.2)
Cerebrovascular disease	1	(3.2)
Respiratory disease	2	(6.5)
Malignancies	6	(19.4)
Autoimmune disease	3	(9.7)
Agammaglobulinemia	1	(3.2)
Length of stay in the hospital, median (range)	33	(5-62)
Length of stay in the ICU, median (range)	25	(5-60)
Ventilation, n (%)	26	(83.9)
Ventilation duration (days), median (range)	18	(4-50)
Dialysis, n (%)	5	(16.1)
Dialysis duration (days), median (range)	17	(4-23)
ECMO, n (%)	5	(16.1)
ECMO duration (days), median (range)	17	(7-20)
Anticoagulation therapy n (%)		
LMWH prophylactic	17	(54.8)
LMWH therapeutic	8	(25.8)
UFH prophylactic	2	(6.5)
UFH therapeutic	2	(6.5)
No anticoagulation therapy	2	(6.5)
Thromboembolic events during ICU stay, n (%)		
CVC thrombosis	4	(12.9)
Clotting of dialysis circuit	2	(6.5)
Clotting of ECMO circuit	3	(9.7)
DVT	2	(6.5)
Stroke	1	(3.2)

Abbreviations: CVC, central venous catheter; DVT, deep vein thrombosis; ECMO, extracorporal membrane oxygenation; ICU, intensive care unit; LMWH, low molecular weight heparin; UFH, unfractionated heparin.

TABLE 2 Patient test results for antiphospholipid antibodies

ID	Thrombotic Complications During ICU Stay	D-dimers	aCL			aβ2GPI		
		ng/mL	aCL IgG (U/mL)	aCL IgM (U/mL)	aCL IgA (U/mL)	aβ2GPI IgG (U/mL)	aβ2GPI IgM (U/mL)	aβ2GPI IgA (U/mL)
1	None	**710**	<3.2	<3.6	<1.4	<11.4	<2.3	<4.0
2	**Clotting dialysis circuit**[a]	**3790**	<3.2	<3.6	2.7	<11.4	<2.3	<4.0
3	**CVC thrombosis**	**1140**	**22.9**	8.0	3.9	<11.4	4.7	<4.0
4	None[a]	**2170**	11.4	8.1	12.4	14.8	4.0	<4.0
5	None	**2990**	9.7	<3.6	2.8	<11.4	<2.3	<4.0
6	**CVC thrombosis**[a]	**2380**	4.8	12.8	3.8	<11.4	<2.3	<4.0
7	**Clotting ECMO circuit**	**3100**	<3.2	<3.6	2.9	<11.4	2.5	<4.0
8	None	**2010**	10.9	<3.6	9.3	<11.4	<2.3	9.8
9	None	**300**	6.1	**467.8**	3.0	**28.8**	**212.8**	<4.0
10	None	**1160**	12.0	<3.6	2.2	<11.4	<2.3	<4.0
11	None	**2690**	4.4	<3.6	3.0	<11.4	<2.3	<4.0
12	None	**2080**	**28.0**	4.4	3.0	<11.4	3.8	<4.0
13	None	**2850**	<3.2	<3.6	<1.4	<11.4	<2.3	<4.0
14	None	**4500**	17.1	3.6	6.7	<11.4	<2.3	<4.0
15	**CVC thrombosis**	**2000**	**36.2**	<3.6	7.1	<11.4	2.7	<4.0
16	**clotting ECMO circuit**	**2940**	11.6	<3.6	3.9	<11.4	4.8	<4.0
17	None	**2600**	4.6	13.3	**74.5**	<11.4	3.4	**90.2**
18	**CVC thrombosis, DVT, stroke**	**5790**	**32.9**	<3.6	3.2	<11.4	3.4	<4.0
19	None	**380**	**27.3**	<3.6	**91.2**	**129.4**	<2.3	**127.1**
20	None	**1450**	**22.4**	8.6	3.7	**33.0**	4.1	<4.0
21	None	**2150**	4.4	6.2	13.6	<11.4	13.3	16.4
22	None	**2310**	3.2	3.6	2.5	<11.4	<2.3	<4.0
23	None	**380**	3.2	3.6	1.6	<11.4	2.8	<4.0
24	**DVT**	**1100**	16.8	7.0	**208.2**	<11.4	2.9	**416.5**
25	None	**1910**	<3.2	<3.6	2.1	<11.4	<2.3	<4.0
26	None	**460**	<3.2	<3.6	2.5	<11.4	<2.3	<4.0
27	None	**2150**	<3.2	5.5	3.3	<11.4	5.8	<4.0
28	None	**570**	<3.2	<3.6	<1.4	<11.4	<2.3	<4.0
ID	Thrombotic complications during ICU stay	D-Dimers	Anticardiolipin antibodies			Antibeta2-glycoprotein I antibodies		
		ng/mL	aCL IgG (U/mL)	aCL IgM (U/mL)	aCL IgA (U/mL)	aβ2GPI IgG (U/mL)	aβ2GPI IgM (U/mL)	aβ2GPI IgA (U/mL)
29	**Clotting ECMO and Dialysis circuit**	**>20 000**	18.4	<3.6	4.1	<11.4	<2.3	<4.0
30	None[a]	**3160**	<3.2	<3.6	3.4	<11.4	<2.3	<4.0
31	None	**450**	3.2	3.6	<1.4	<11.4	<2.3	<4.0

Note: All positive results are written in bold type.

−, negative; +, positive; aCL, anticardiolipin antibodies; aPS/PT, anti-prothrombin/phosphatidyl serine antibodies; APTT, activate partial thromboplastin time; aβ2GPI, antibeta2-glycoprotein I antibodies; CVC, central venous catheter; dRVVT, dilute Russell's viper venom time; DVT, deep vein thrombosis; ECMO, extracorporal membrane oxygenation; ICU, intensive care unit; ID, patient identification; LAC, lupus anticoagulant.

[a]Patient died during stay in ICU.

aCL IgG was positive. One LAC-negative patient was single positive for aCL IgG (patient 12) and one LAC-negative patient was double positive for aCL IgG and aβ2GPI IgG (patient 20). Three patients had LAC positivity and aCL IgG (patient 3, 15, 18). Sixteen of 21 (76%) patients were single LAC-positive. Two patients were triple-positive (patients 9 and 19).

aPS/PT		LAC			Repeat Testing (1 mo After First Occasion)				
aPS/PT IgG (U/mL)	aPS/PT IgM (U/mL)	LAC dRVVT	LAC APTT	LAC final conclusion	LAC dRVVT	LAC APTT	LAC final conclusion	aCL IgG (U/mL)	aβ2GPI IgG (U/mL)
<8.4	<10.8	+	+	**Positive**	–	–	Negative		
<8.4	<10.8	–	–	Negative					
<8.4	23.6	+	+	**Positive**	–	–	Negative	11	
10.1	21.2	+	+	**Positive**					
<8.4	11.4	–	–	Negative					
130.5	<10.8	+	+	**Positive**					
13.0	<10.8	+	–	**Positive**	–	–	Negative		
11.4	<10.8	–	–	Negative					
<8.4	15.5	+	–	**Positive**					
13.5	<10.8	–	–	Negative					
<8.4	<10.8	–	–	Negative					
<8.4	11.4	–	–	Negative					
<8.4	**32.3**	+	–	**Positive**					
10.81	**32.7**	+	–	**Positive**					
<8.4	18.9	–	+	**Positive**					
<8.4	<10.8	–	–	Negative					
27.3	26.9	+	–	**Positive**	–	–	Negative		
<8.4	<10.8	+	–	**Positive**	–	–	Negative	7.4	
72.1	12.1	+	+	**Positive**	+	+	**Positive**	13.8	**49.1**
<8.4	<10.8	–	–	Negative				2.6	19.9
<8.4	13.4	+	+	**Positive**	–	–	Negative		
<8.4	<10.8	–	–	Negative					
<8.4	<10.8	+	+	**Positive**					
<8.4	**60.1**	+	–	**Positive**					
<8.4	<10.8	+	+	**Positive**	–	–	Negative		
<8.4	<10.8	+	+	**Positive**	–	–	Negative		
<8.4	**38.8**	+	+	**Positive**					
<8.4	<10.8	–	–	Negative					

Anti-PS/PT antibodies		Lupus anticoagulant			Repeat testing (1 mo after first occasion)				
aPS/PT IgG (U/mL)	aPS/PT IgM (U/mL)	LAC dRVVT	LAC APTT	LAC final conclusion	LAC dRVVT	LAC APTT	LAC final conclusion	aCL IgG (U/mL)	aβ2GPI IgG (U/mL)
14.7	<10.8	+	–	**Positive**	–	–	Negative		
285.9	<10.8	+	+	**Positive**					
<8.4	<10.8	+	+	**Positive**					

Seven of nine patients with thrombotic complications had at least one criterion aPL-positive, and four with single LAC positivity. Sixteen of 22 patients without thrombotic complications showed positivity for at least one criterion aPL, 13 with single LAC positivity. The two triple-positive patients had no thrombotic complications.

TABLE 3 Patient test results for lupus anticoagulant

Patient ID	Results LAC dRVVT Screen N ratio	Results LAC dRVVT Screen Mix N ratio	Results LAC dRVVT Screen/Confirm N ratio	Results LAC dRVVT Conclusion	Results LAC APTT Screen N ratio	Results LAC APTT Screen Mix N ratio	Results LAC APTT Delta (s)[a]	Results LAC APTT Conclusion	LAC Final Conclusion
Cutoff	**1.39**	**1.10**	**1.10**		**1.33**	**1.12**	**8.0**		
1	**1.86**	**1.42**	**1.27**	+	**1.91**	**1.59**	**24.7**	+	**Positive**
2	1.31			−	**1.86**	**1.42**	1.5	−	Negative
3	**1.51**	**1.21**	**1.14**	+	**1.92**	**1.42**	**19.1**	+	**Positive**
4	**2.63**	**1.60**	**1.61**	+	**1.53**	**1.13**	**11.6**	+	**Positive**
5	1.25			−	0.95			−	Negative
6	**1.92**	**1.29**	**1.30**	+	**3.49**	**2.28**	**21.3**	+	**Positive**
7	**2.49**	**1.64**	**1.59**	+	1.10			−	**Positive**
8	1.07			−	1.16			−	Negative
9	**1.44**	**1.21**	**1.10**[b]	+	**1.41**	**1.23**	7.4	−	**Positive**
10	0.94			−	1.00			−	Negative
11	1.29			−	1.08			−	Negative
12	1.36			−	1.03			−	Negative
13	**1.65**	**1.24**	**1.23**	+	0.98			−	**Positive**
14	**1.94**	**1.43**	**1.42**	+	1.13			−	**Positive**
15	1.39			−	**1.45**	**1.17**	**8.9**	+	**Positive**
16	1.27			−	0.87			−	Negative
17	**1.55**	**1.29**	**1.26**	+	1.22			−	**Positive**
18	**1.63**	**1.37**	**1.32**	+	1.04			−	**Positive**
19	**3.15**	**1.84**	**1.71**	+	**2.08**	**1.41**	**23.7**	+	**Positive**
20	1.35			−	0.95			−	Negative
21	**1.71**	**1.41**	**1.28**	+	**1.72**	**1.36**	**18.6**	+	**Positive**
22	1.21			−	1.09			−	Negative
23	**1.78**	**1.31**	**1.16**	+	**1.73**	**1.38**	**22.9**	+	**Positive**
24	**1.42**	**1.19**	**1.10**[b]	+	1.23			−	**Positive**
25	**1.96**	**1.43**	**1.35**	+	**1.40**	**1.25**	**25.2**	+	**Positive**
26	**1.68**	**1.28**	**1.19**	+	**1.69**	**1.42**	**18.5**	+	**Positive**
27	**1.56**	**1.32**	**1.14**	+	**1.38**	**1.29**	**14.9**	+	**Positive**
28	1.29			−	0.97			−	Negative
29	**1.46**	**1.21**	**1.17**	+	**4.04**	**2.13**	6.1	−	**Positive**
30	**1.87**	**1.73**	**1.71**	+	**2.45**	**2.07**	**49.6**	+	**Positive**
31	**2.38**	**1.27**	**1.16**	+	**3.20**	**1.17**	**30.0**	+	**Positive**

All positive results are written in bold type (in-house calculated cutoff values on 120 normals).

Abbreviations: −, negative; +, positive; APTT, activate partial thromboplastin time; dRVVT, dilute Russell's viper venom time; ID, patient identification; LAC, lupus anticoagulant; N ratio, normalized ratio; s, seconds.

[a]Confirmatory step for APTT (Staclot LA) is expressed as a difference in clotting time between two APTTs with and without hexagonal phase phosphatidyl ethanolamine.

[b]dRVVT Screen Mix/Confirm Mix N ratio > cutoff (0.92).

Repeat testing of positive aPL results 1 month after the first occasion could be performed in part (11/31) of the patient population. Samples after 1 month were not available from all patients.

LAC was repeated in 10 of 21 patients that were LAC-positive during the first period of testing. Nine of ten patients were LAC-negative on the second occasion. One (patient 19) of the two triple-positive patients was included in the repeat testing series and showed persistent LAC positivity after 1 month. However, the aCL IgG originally positive around the cutoff value was negative by repeat testing. In this patient, aβ2GPI IgG persisted positive by repeat testing, albeit with a lower titer. Repeat testing of borderline positive aCL IgG and low positive aβ2GPI IgG (patient 20) was negative on the second testing. Seven patients were single LAC-positive on the first occasion, two patients (patients 3 and 18) were combined positive for LAC and aCL IgG (borderline positive). These patients also tested negative for aCL IgG by repeat testing. Four of the nine patients with negative LAC during repeat testing showed thrombotic complications.

The aPS/PT IgG were positive in three patients (patients 6, 19, 30); one patient with single LAC positivity and thrombosis, one triple-positive patient, and one single LAC-positive patient, both without thrombotic complications, respectively. aPS/PT IgM were positive in four patients (patients 13, 14, 24, 30), three with single LAC positivity and no thrombotic complications, and one with single LAC positivity and DVT, respectively.

aCL IgA and aβ2GPI IgA was combined positive in three patients (patients 17, 19, 24), all associated with LAC. One patient had DVT.

All patients had elevated D-dimers (Table 2).

4 | DISCUSSION

The incidence of both arterial and venous thromboembolism is high in COVID-19 patients, and laboratory markers may help in raising suspicion of underlying thrombotic problems.[33-36] Changes in coagulation parameters detecting the procoagulant state in COVID-19 patients associated with poor clinical outcome were reported.[3-6] Simple, and for most institutions available, laboratory markers such as platelet count, D-dimers, prothrombin time, and fibrinogen seemed relevant for laboratory monitoring for COVID-19 related coagulopathy in addition to clinical assessment.[36,37] The hemostatic changes observed in COVID-19 are previously also been shown in association with other coronaviruses,[38] and some viruses are known to activate the coagulation system.[39]

Clinical experience learns that the hypercoagulable state in critically ill COVID-19 patients comprise diverse types of thromboembolic complications that need adequate anticoagulant therapy.[5,36,40-42] Triggered by the hypercoagulable state of these patients and the high incidence of thrombosis, involvement of aPL has been suggested and reports have been published on measurement of aPL in COVID-19 patients.[5,7-9,42]

Zhang et al described three patients with multiple cerebral infarctions and presence of aCL IgA and aβ2GPI IgG and IgA positivity, measured on one occasion, without details on the titers provided. No LAC was detected in these patients.[7] The three patients fulfilled the clinical criteria for APS,[11] but had also other comorbidities associated with thrombosis.[7]

Harzallah et al tested 56 COVID-19 patients for aPL, and found 45% LAC positive, and 10% either aCL or aβ2GPI IgG or IgM positive, of which three were associated with LAC. Titers of aCL or aβ2GPI were not reported, and no details were provided on whether LAC was positive in the APTT and/or dRVVT test system and associated thrombosis was not mentioned.[9]

Bowles et al described 35 patients in detail, and detected 91% of 35 patients positive for LAC in a workout for prolonged APTT.[8] Six of 35 patients (18%) were positive in the APTT system only. aCL or aβ2GPI were not measured and only two patients had thrombosis.[8]

Helms et al tested for LAC in 57 patients with a thrombotic event during their stay at the ICU or when a coagulation disorder was suspected based on prolonged APTT.[5] LAC testing did not include a confirmatory step for the APTT system, and LAC was considered positive based on the dRVVT test system results only. They observed 88% positives for LAC.[5] aCL and aβ2GPI were not tested, but one patient seemed to have aCL IgM (48 MLP [IgM phospholipid units]) positive before the COVID-19 infection. The number of LAC-positive patients with confirmed thrombosis is not reported. In the overall population, 64 of 150 patients (43%) had thrombosis.[5]

In two of the four published studies, COVID-19 patients were not tested for aCL and aβ2GPI.[5,8] If not all criteria aPL are measured, no antibody profiles could be done that proved to be useful because combining the aPL may improve risk assessment.[10,11,43-45] Combined positivity for LAC, aCL, and aβ2GPI antibodies (ie, triple positivity) has been shown to be associated with a high risk of both a first thrombotic event and recurrence.[46-48] Double-positive (LAC-negative) patients are at lower risk than triple-positive patients, and single-positive patients are less likely to develop APS-related clinical symptoms.[10,44] Isolated positivity for LAC is often observed in absence of clinical symptoms, in elderly patients, on a first occasion not confirmed after 12 weeks.[44,49,50] An isolated LAC is an independent risk factor for myocardial infarction and ischemic stroke, however.[51,52]

The aPL antibody profiles demonstrated in COVID-19 patients have a low-risk profile for thrombosis. Studies that included aCL and aβ2GPI showed mainly single LAC-positive patients.[9] In the study of Zhang et al,[7] considering the criteria aPL,[10] the three patients described show single positivity for aβ2GPI IgG. In our study population, 23% (7/31) of patients had aCL and/or aβ2GPI, slightly higher compared with the study of Harzallah et al.[9] In previously reported cohorts,[7,9] aCL or aβ2GPI titers were not reported and cannot be valued against the high titers that are required according to the guidelines.[11,20] In our cohort, the titers of aCL IgG ranged from 22.4 to 36.2 U/mL, although our cutoff value corresponds to the 99th percentile[20] experience shows that these titers are "low" positive for the solid phase test system we used.[29,30] Moreover, values around the cutoff value (three of the five positive samples) should be interpreted with care.[20] Only triple-positive patients demonstrated higher titers (patients 9 and 19). All patients with high titers aCL or aβ2GPI did not have thrombotic complications so far.

As far as interpretable and based on available results in previous studies,[5,7-9] in none of the patients was triple positivity demonstrated. In our patient cohort, only two patients were triple positive, of whom none showed thrombotic complications. Although the incidence of aPL in our cohort was high with 74% of patients positive for at least one criterion aPL, the majority of patients showed a low-risk profile: 16 single LAC positives, one sample with single aCL, one sample with double positivity, and three samples with LAC and aCL positivity.

In previous studies,[5,7-9] the association of aPL and thrombosis is strongly highlighted, but it is unclear whether all these patients were prophylactically anticoagulated. In our cohort of ICU patients, who tested all positive for D-dimers, we observed no strong association of aPL and thrombotic complications. Among the described thrombotic complications in COVID-19 patients (clotting of CVC, clotting of dialysis filters, stroke, DVT, and ischemic limbs), we mainly observed CVC and circuit device clotting; one of the nine patients with thrombotic complications showed stroke and another patient showed DVT.[40,53] There was no relationship between D-dimers and aPL, thrombosis, or outcome. In patients with thrombosis, 67% showed positivity for aPL; in the patients without thrombosis, 72% tested positive for at least one aPL. During ECMO treatment, all five patients received UFH. Importantly, in our cohort, the majority of patients were treated with heparins to prevent thrombotic complications. This supports that patients should be anticoagulated because coagulopathy is one of the key features associated with poor outcomes.[33,36]

Regarding the isotype, in our study, one triple-positive patient (patient 9) was positive for aPL IgM. In the patient population described by Harzallah et al, maximally 10% were IgM positive and all aCL/aβ2GPI-positive patients were associated with LAC. In our study population, we observed two patients with aCL/aβ2GPI not associated to LAC, all of whom were IgG positive. This is in line with what we illustrated in a recent multicenter study: that isolated positivity of IgM was rare in thrombotic APS and that it was not an independent risk factor for thrombosis.[18]

Despite aCL and aβ2GPI IgA not being included in the current classification criteria,[10] we tested for IgA. Zhang et al found all three patients (with cerebral infarctions) positive for both aCL and aβ2GPI IgA without LAC positivity.[7] We observed three patients positive for both aCL and aβ2GPI IgA (high titers), associated with LAC, of whom one patient had DVT. In two of the three patients, aCL/aβ2GPI IgG and IgM were negative, which is relatively high (2/31, 6%) compared with an APS setting where isolated IgA positivity is rare in patients with clinical manifestations of APS.[31] In most cases with major APS manifestations (ie, thrombosis), IgA aPL are usually found in association with IgG and/or IgM.[21] The number of patients positive for IgA aPL (n = 3) compared with IgG (n = 8) and IgM (n = 2), is comparably low.

In this COVID-19 cohort, the role of IgA is unclear, without added value on top of the current classification criteria, equally as previously illustrated in APS.[10,21,30]

Amongst noncriteria aPL, aPS/PT is a group of aPL that merit attention, based on recent literature describing their association with thrombosis.[22,23] aPS/PT antibodies are strongly associated with LAC and frequently present in APS patients.[49,54] In our COVID-19 cohort, we observed aPS/PT IgM positivity in 25% (4/16; patients 13, 14, 24, 27) of the single LAC positives, of which one patient suffered from DVT. Three patients were positive for aPS/PT IgG (patients 6, 19, 30), two with single LAC positivity, of which one had CVC thrombosis and two (one with triple positivity) had no thromboembolic complications. The association of LAC and aPS/PT seems lower than expected based on results in APS patients. Today, aPL against only two plasma proteins, β2GPI and prothrombin, are found frequently enough in APS patients to attribute them a pathophysiological role. In the absence of aβ2GPI, LAC positivity signifies a β2GPI-independent LAC whose association with thrombosis is uncertain.[44] The single LAC positivity frequently observed in COVID-19 patients might be explained by LAC activity through other cofactors. We can speculate that in these single LAC-positive patients (hence aβ2GPI negativity, and also aPS/PT negativity), aPL binding through other cofactors, such as complement C4 or factor H, may be responsible for the LAC positivity. Additionally, the role of complement activation and cytokine storm has been described in COVID-19 and may play a role in the microvascular injury and organ dysfunction.[10,42,55]

In the published studies, there is no information on LAC (or other aPL) positivity before COVID-19 infection. In the context of APS, previous studies illustrated that in asymptomatic carriers the number of events was much lower in double and single LAC positives compared with triple positives.[48,56] Double-positive (LAC-negative) patients were at lower risk than triple-positive patients, and single-positive patients are less likely to develop APS-related clinical symptoms.[10,44] If we assume that all patients testing positive for LAC during COVID-19 infection were asymptomatic carriers, we should also assume they are less expected to develop aPL-related thrombosis. Also, in our study, we did not test for aPL in the majority of patients before COVID-19 infection. At COVID-19 infection, one patient was diagnosed as asymptomatic carrier with persistent positive single LAC and did not develop thrombosis during infection but had severe comorbidity and died during his stay at the ICU. The two triple-positive carriers in our cohort did not develop thrombosis at the time of this writing.

All aPL analyses were performed during the acute phase, which is discouraged in the guidelines because of possible interferences with the test result.[24] Single LAC positivity is a common finding in all COVID-19-related studies measuring aPL. Isolated LAC may also be the consequence of the complicated methodology of phospholipid-dependent coagulation tests that are prone to interferences.[12,24,26] In some of the published reports, there is concern about the methodology because most of these critically ill patients have raised levels of CRP, that may results in false-positive LAC[26] In some publications, we can rule out this reason for false positivity,[5,8] but in others we cannot.[9] One of the major drawbacks of LAC testing is also the interference of anticoagulant therapy.[28] COVID-19 patients are treated with heparins,[36] but interference of heparins is probably not a real issue here because reagents dedicated for LAC testing contain heparin neutralizers, and LAC analysis is reliable if antiXa levels of heparins are within the therapeutic range.[28] Although we also tested during the acute phase in our observational study, we are confident of not having false-positive LAC because we checked for CRP and antiXa levels, and nevertheless observed 52% (16/31) single LAC-positive patients.

A major drawback of all COVID-19-related studies on aPL is the lack of confirmed positivity of aPL after 3 months.[10] Positive results of LAC, aCL, or aβ2GPI need to be confirmed on a second occasion after 12 weeks to confirm persistent positivity.[10] We had the occasion to retest some patients at a second time point, at 1 month distance from the first testing period. All but one patient retested for

LAC became negative, for aCL IgG all retested positive patients were negative. It is noteworthy that the retested triple-positive patient turned into negative for one aPL (aCL IgG) after 1 month. Transient antibodies have been described in infectious diseases or drugs and are thought of not being of clinical significance; therefore, retesting was originally meant to avoid overdiagnosis of APS patients that were not persistently positive.[10,11] Some studies demonstrated that aPL, with properties similar to those found in patients with APS, can be induced by immunization with β2GPI-like PL-binding viral and bacterial products. However, it is not certain that these aPL antibodies are pathogenic, and the clinical significance remains unknown. Infection-induced aPL is transient in some patients, and in some individuals they persist and can be associated with thrombosis.[15,57] Infectious agents are triggers for the formation of aPL and molecular mimicry between structures of bacteria or viruses and β2GPI-derived amino acid sequences are thought to contribute to the formation of autoantibodies.[58] But only with the appropriate genetic background or following secondary triggers do these antibodies become pathogenic. Triggers probably push the hemostatic balance in favor of thrombosis and might include environmental factors such as infection.[59]

Limitations of our study are the small patient population and the limited number of patients that were retested on a second occasion on distance from the first testing.

In summary, the observations in our study support the finding of frequent single LAC positivity in the acute phase of the COVID-19 infection, but not clearly related to thrombotic complications. Albeit our study population is small, triple-positive patients are rare, and titers of aCL and aβ2GPI are high only in the minority of patients. LAC positivity does not correspond with aPS/PT as we observe in APS. Repeat testing in a limited number of patients suggests that most of the aPL are transient.

We can conclude that it is clear that alterations in the hemostatic balance in COVID-19 patients is strongly disturbed and contribute to a high prothrombotic status, justifying the use of anticoagulant therapy. The hypercoagulability observed in COVID-19 patients is probably multifactorial, and not clarified today. Inflammation is closely associated to thrombosis and dysregulation in the coexistence and interplay of hemostatic and inflammatory mediators can result in clinical manifestations of disease, including thrombosis.[60] On top of all the interest in this new virus, the association between viral and bacterial infections with high inflammatory state and the incidence of thromboembolic events is not a new finding.[61]

Today, it is premature to conclude there is a contribution of aPL to thrombosis in these patients. Further studies are needed to further unravel the role of aPL in COVID-19 patients and the relation with thrombosis. The presence of aPL should be interpreted with appropriate reservations and we should be conscious that preanalytical and analytical variables can affect test results, especially for LAC. Large well-designed clinical studies are required before clear conclusions can be made on routine testing of aPL in COVID-19 patients. Also, we have to follow-up on the first measurements we now have available, to evaluate the persistence of the positive aPL in these patients. An international registry of aPL measurement and follow-up should be very helpful in understanding the role of aPL in thrombotic complications in COVID-19 patients.

CONFLICT OF INTEREST

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

Katrien M. J. Devreese, Harlinde Peperstraete, and Dominique Benoit conceived the study. Katrien M. J. Devreese wrote the manuscript. Eleni A. Linskens collected the data. Harlinde Peperstraete and Dominique Benoit revised the clinical data. All authors revised and approved the manuscript.

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How to cite this article: Devreese KMJ, Linskens EA, Benoit D, Peperstraete H. Antiphospholipid antibodies in patients with COVID-19: A relevant observation?. *J Thromb Haemost*. 2020;18:2191–2201. https://doi.org/10.1111/jth.14994