"True" Antiphospholipid Syndrome in COVID-19: Contribution of the Follow-up of Antiphospholipid Autoantibodies

Robin Arcani, MD, MSc[1,2] Raphaël Cauchois, MD, MSc[1,2] Pierre Suchon, MD, PhD[3] Samuel Weber, MD[4] Rodolphe Jean, MD[1] Pierre-André Jarrot, MD, PhD[1,2] Louise Rey, PharmD[4] Geoffroy Venton, MD, PhD[5] Marie Koubi, MD, MSc[1] Romain Muller, MD, MSc[1,2] Daniel Bertin, PharmD, PhD[4] Jean-Louis Mège, MD, PhD[4,6] Gilles Kaplanski, MD, PhD[1,2] Nathalie Bardin, PharmD, PhD[2,4]

[1] Department of Internal Medicine and Clinical Immunology, CHU La Conception, Assistance Publique-Hôpitaux de Marseille (AP-HM), Marseille, France
[2] Aix-Marseille University, INSERM, INRAE, C2VN, Marseille, France
[3] Hematology Laboratory, CHU La Timone, Assistance Publique-Hôpitaux de Marseille (AP-HM), Marseille, France
[4] Service d'Immunologie, Pôle de Biologie, Hôpital de la Conception, Assistance Publique-Hôpitaux de Marseille (AP-HM), France
[5] Department of Hematology and Cellular Therapy, CHU La Conception, Assistance Publique-Hôpitaux de Marseille (AP-HM), Marseille, France
[6] Aix-Marseille University, IRD, MEPHI, IHU-Méditerranée Infection, Marseille, France

Address for correspondence Robin Arcani, MD, MSc, Department of Internal Medicine and Clinical Immunology, CHU La Conception, Assistance Publique-Hôpitaux de Marseille (AP-HM), 147 Boulevard Baille, 13005 Marseille, France (e-mail: robin.arcani@ap-hm.fr).

Semin Thromb Hemost 2023;49:97–102.

COVID-19 has led to more than 5 million deaths to date.[1] COVID-19 presentation can range from a simple asymptomatic viral infection to acute respiratory distress syndrome (ARDS)[2]; 15 to 20% of patients develop severe pneumonia and experience coagulopathy disorders. Severe COVID-19 occurs in the context of hyperinflammation that could potentially generate autoimmune disease.[3] We and others have shown the presence of antiphospholipid (aPL) autoantibodies in patients with COVID-19.[4] However, there remains contention if these aPLs represent transient antibodies associated with infection, or "true" aPL reflective of potential autoimmune-associated coagulopathy, namely, antiphospholipid syndrome (APS).[5,6] Thus, only if they can be shown to persist for at least 12 weeks, can they be considered essential markers for the diagnosis of APS.[7,8]

We previously reported that aPLs are highly and independently associated with disease severity particularly in patients requiring hospitalization in intensive care units (ICUs).[4,9] However, the occurrence of "true" APS is still debated in COVID-19.[5,6] The link between the persistent presence of aPLs and the appearance of thrombosis during COVID-19 or during disease follow-up is not clearly established.[10–12]

In this context, we studied the occurrence of APS after COVID-19 in patients who were positive for aPLs. For this purpose, we analyzed the clinical characteristics of the patients, including thrombosis, according to the persistence of aPLs from the acute phase of the disease to a follow-up at 12 weeks.

We retrospectively included all consecutive adult patients hospitalized with a laboratory-confirmed SARS-CoV-2 infection assessed by RT-PCR on nasopharyngeal swabs and systematically screened them for conventional aPLs autoantibodies (lupus anticoagulant [LA], IgG/IgM anticardiolipin [aCL], and IgG/IgM anti-β2-glycoprotein I [aB2GPI]) between January and April 2021 in two COVID-19 units (La Conception, University Hospital of Marseille, France). All patients positive for at least one conventional aPL were given an appointment and were examined 12 weeks after the first dosage of the aPL autoantibodies. A second dosage of conventional aPL autoantibodies was performed at the 12-week follow-up visit. Patients were defined as having persistent

article published online November 6, 2022

Issue Theme Maintaining Hemostasis and Preventing Thrombosis in COVID-19—Part IV; Guest Editors: Emmanuel J. Favaloro, PhD, FFSc (RCPA), Leonardo Pasalic, FRCPA, FRACP, PhD, and Giuseppe Lippi, MD

Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

DOI https://doi.org/10.1055/s-0042-1758118.
ISSN 0094-6176.

aPL when aPL autoantibodies were positive two times at least 12 weeks apart.

Concentrations of aPLs autoantibodies were measured with commercially available ELISA. aCL (IgG and IgM) and aB2GPI (IgG and IgM) were measured with CardiolisaTheradiag (Marne-la-Vallée, France) and Orgentec Diagnostica (Mainz, Germany), respectively. Positive cut-offs were 15 U/mL for aCL and 8 U/mL for aB2GPI according to the manufacturers' recommendations and on-site validation. To avoid nonspecific binding issues, each positive sample was duplicated, and serum nonspecific background of uncoated well was subtracted from the measured optical density of the coated well. LA was determined as recommended by International Society of Thrombosis and Haemostasis (ISTH)[8] using two clotting times: partial thromboplastin time–lupus anticoagulant (PTT-LA) by Diagnostica Stago (Asnières-sur-Seine, France) and diluted Russell viper venom time (dRVVT) by Hyphen BioMed (Neuville-sur-Oise, France). PTT reagent comprised a single LA screening reagent. The Rosner index (RI) was calculated and was considered positive when RI was greater than 15%. The dRVVT results were expressed with a normalized ratio (NR) (positive value, >1.2). Patients were considered positive for LA when both PTT-LA and dRVVT were positive. Screening for aPL was performed at the time of admission to the unit; so, the patients were mainly not anticoagulated. In case of patients under anticoagulation, PTT was performed if anti-Xa activity was less than 0.15. The dRVVT was not performed if anti-Xa activity was greater than 0.8. In case of patients under vitamin K antagonists, PTT was performed if the international normalized ratio (INR) was less than 3, and the dRVVT was performed in a mix with normal pooled plasma if INR was between 2 and 3. The two tests were not performed if INR was greater than 3. LA was not assessed in patients under direct oral anticoagulants.

This study was approved by the Institutional Review Board of Assistance Publique - Hôpitaux de Marseille (GDPR number PADS21-4) and conducted according to the declaration of Helsinki.

Quantitative data were compared using Student's *t*-test or the Mann–Whitney *U*-test; qualitative data were compared with the chi-square or Fisher's exact test when appropriate. Multivariate logistic regression was applied to identify independent risk factors associated with ICU transfer or death using variables with a *p*-value less than 0.15 by univariate analysis. The tests were two-sided. *p*-Values less than 0.05 were considered significant.

Hospitalized COVID-19 patients ($n = 238$) were analyzed as presented in ►**Fig. 1**; 185 (77.7%) were screened for conventional aPLs, and 66 (35.7%) were positive for at least one aPL (►**Fig. 1**). The main characteristics of the cohort are presented in ►**Table 1**. The mean age was 64.1 ± 16.4 years (range: 19–103) with 109 male patients (58.9%). All patients were treated with heparin during COVID-19 according to the current recommendations from the French Society of Critical Care[13] and received usual thromboprophylaxis for 14 days following discharge. There were 36 of 167 eligible patients (21.6%) transferred to the ICU, and 7 patients out of 167 eligible patients (4.2%) required mechanical ventilation; 58 patients (31.4%) needed supplemental oxygen flow greater than 6 L/minute. Sixteen patients (8.6%) died during hospitalization. Ten patients (5.4%) experienced at least one thrombosis during hospitalization (four strokes, four deep venous thromboses, one central catheter–related thrombosis, and one coronary thrombosis) despite thromboprophylaxis.

LA was positive in 39 patients (21.1%), aCL autoantibodies were positive in 28 patients (15.1%; 22 aCL IgG and 6 aCL IgM), and aB2GPI was positive in 15 patients (8.1%; 5 aB2GPI IgG and 10 aB2GPI IgM). There were 53 patients (28.6%) with only one aPL; 10 patients (5.4%) had two aPLs, and three patients (1.6%) were triple-aPL positive. After multivariate logistic regression, we found that the presence of aPL was associated only with ICU transfer (odds ratio [OR]: 2.75, 95% confidence interval [CI]: 1.27–6.01, $p = 0.01$). We also found that the age (OR: 1.049, 95% CI: 1.003–1.097, $p = 0.038$) and the presence of an active cancer (OR: 4.55, 95% CI: 1.27–16.34, $p = 0.020$) were independently associated with death.

Clinical data were compared between 66 aPLs patients (59.1% with LA, 33.3% with aCL IgG, 9.1% with aCL IgM, 7.6% with aB2GPI IgG, and 15.2% with aB2GPI IgM) and 119 patients without aPLs. A higher rate of transfer to the ICU (32.7 vs. 16.1%, $p = 0.014$) was observed in patients with positive aPLs. No significant differences were observed regarding the mortality of patients with aPLs versus those without aPLs (7.6 vs. 9.2%, $p = 0.67$). During the acute phase of the disease, there was a higher rate of thrombosis in patients with aPL, but this was not statistically significant (9.1 vs. 3.4%, $p = 0.17$).

Of the 66 aPLs patients, 45 patients were followed up for 12 weeks. The mean time between the two aPLs tests was 14.6 ± 3.4 weeks. Thirteen patients (28.9%) had at least one persistent aPL with a single positivity in 69.2%, double positivity in 15.4%, and triple positivity in 15.4%. Most patients (10/13, 76.9%) had persistently positive aCL (7 patients with aCL IgG and 3 patients with aCL IgM), 5 patients (38.5%) had persistent LA, and 4 patients (30.8%) had persistently positive aB2GPI.

A comparison between patients with persistent positive aPLs ($n = 13$) to 151 patients with negative aPL (including 32 patients with transient aPLs) showed that patients with persistent positive aPLs were more frequently female (69.2 vs. 34.4%, $p = 0.013$) and less frequently had diabetes (0 vs. 27.8%, $p = 0.027$). Interestingly, they had more frequent histories of cardiovascular diseases (30.8 vs. 9.3%, $p = 0.039$). Importantly they had a significantly higher occurrence of thrombotic events (23.1 vs. 4%, $p = 0.025$) than negative aPL patients (►**Table 2**). Of the 13 patients with persistent aPLs, two had a thrombosis during hospitalization for COVID-19 (two strokes); one patient developed a thrombosis during the follow-up (portal vein thrombosis), and no thrombosis was observed during the follow-up of patients with transient aPLs ($n = 32$). Therefore, out of 45 patients with two screenings for aPLs 12 weeks apart, 3 patients (6.7%) fulfilled the APS/Sydney criteria.[7]

Of the 10 patients who experienced thrombosis during the acute phase of COVID-19, two of them developed a "true"

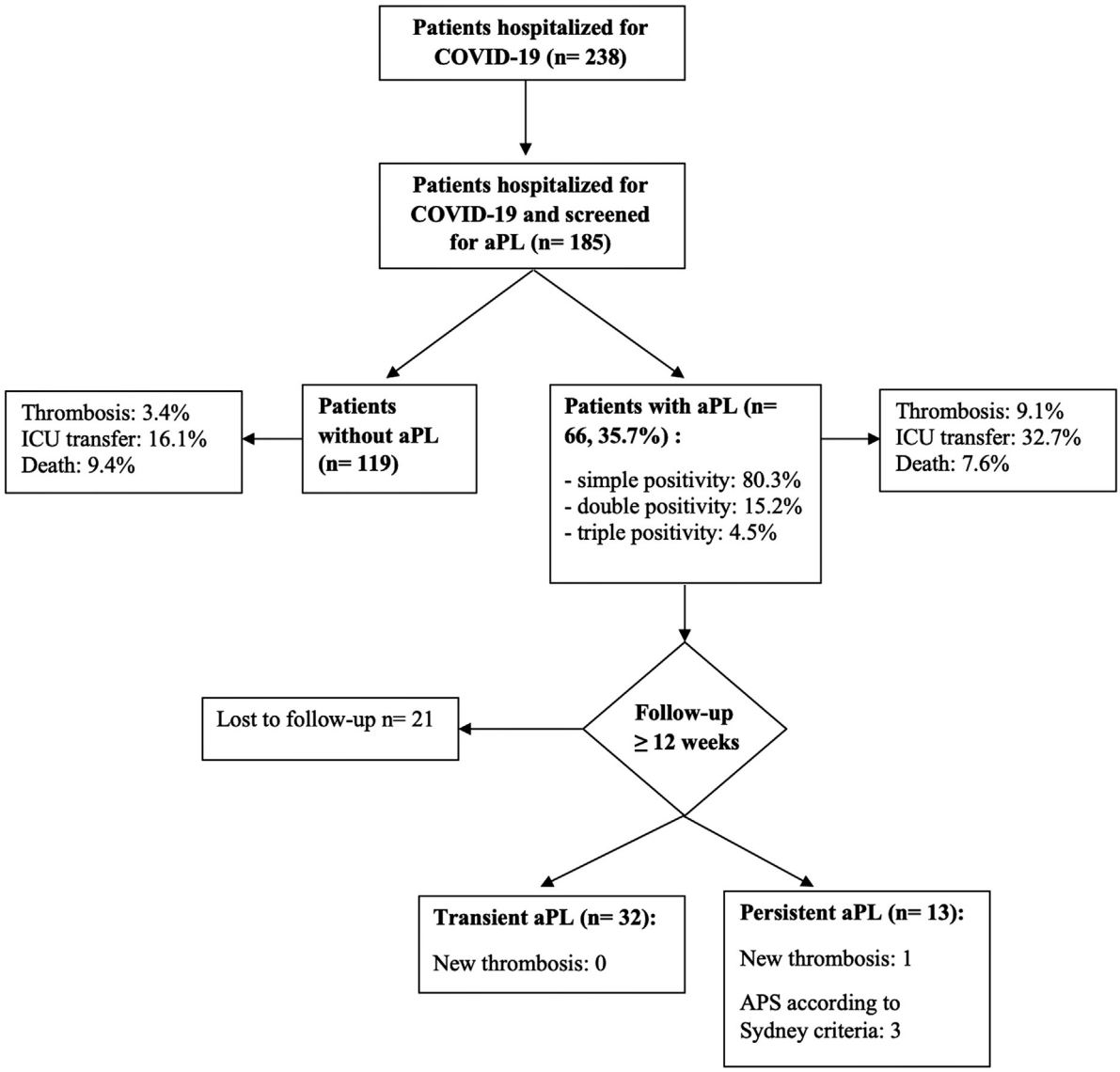

Fig. 1 Flow chart of the cohort.

APS (20%). Of the 185 patients screened for aPLs, the incidence of APS was 1.6%. Assuming that the estimated prevalence of APS in the general population is 50 per 100,000 inhabitants,[14] the relative risk of developing APS could be estimated (1.6/0.05). This indicates that the relative risk of developing APS in COVID-19 patients is 32.

The high prevalence of aPLs and thrombosis reported in COVID-19, a pathology with a strong thromboinflammatory response, has questioned the risk of developing "true" APS, a prothrombotic autoimmune disease, in COVID-19.[5,6] Although APS is a rare autoimmune disease, we are able to show here a higher risk of developing APS during COVID-19, although the absolute event rate for APS was only 1.6%.

We also confirm the high prevalence of aPLs in COVID-19 and their association with severe disease. According to the APS criteria, we checked for aPL persistence 12 weeks apart and established persistence in almost 30% of initially positive COVID-19 patients. Very few groups have reported a follow-up of aPL testing in patients with COVID-19. Gil-Etayo et al[12] reported no decrease in aPL prevalence during follow-up, and Vollmer et al[11] showed that LA was transient and that no patients had a new episode of thrombosis. Our results appear quite similar with a clear decrease in LA positivity (from 39 patients to 5 patients).

We found that persistent aPLs were associated with thrombotic events as defined for the APS diagnosis[7] in three patients from the time of hospitalization for COVID-19 to the end of the follow-up, that is, 1.6% of patients corresponded to a "true" APS. Considering a 0.05% prevalence of APS in the general population,[14] our results suggest that the relative risk of developing APS is significantly increased in patients with COVID-19. Vollmer et al[11] found similar results with an APS diagnosed in 7 of 42 patients after a 12-week follow-up. However, we observed no new thrombosis after patient discharge with transient aPLs during the follow-up. There was only one thrombotic event in a patient with persistent aPLs. The risk of thrombosis may be relatively low after discharge even in patients with aPLs during COVID-19.

Table 1 Main characteristics of the studied population

Characteristics	Whole cohort ($n = 185$)	aPL− ($n = 119$)	aPL+ ($n = 66$)	p-Value[a]
Age (mean ± SD)	64.1 ± 16.4	63.6 ± 16.9	65 ± 15.6	0.57
Gender (male)	109 (58.9)	71 (59.7)	38 (57.6)	0.78
Length of hospitalization	12.2 ± 11.7	13.7 ± 13.6	9.6 ± 6.5	**0.0064**
Comorbidities				
- Obesity	38/175 (21.7)	18/109 (16.5)	20 (30.3)	**0.032**
- Pregnancy	2 (1.1)	1 (0.84)	1 (1.5)	0.67
- Diabetes	58 (31.4)	33 (27.7)	25 (37.9)	0.15
- Hypertension	79 (42.7)	49 (41.2)	30 (45.5)	0.57
- Chronic lung disease	28 (15.1)	17 (14.3)	11 (16.7)	0.26
- Dyslipidemia	34 (18.4)	22 (18.5)	12 (18.2)	1
- Cardiovascular disease	21 (11.4)	8 (6.7)	13 (19.7)	**0.0077**
- History of thromboembolic venous disease	6 (3.2)	1 (0.84)	5 (7.6)	**0.013**
- Autoimmune disease	5 (2.7)	1 (0.84)	4 (6.1)	**0.036**
- Chronic kidney failure	14 (7.6)	7 (5.9)	7 (10.6)	0.24
- Immunosuppression	15 (8.1)	7 (5.9)	8 (12.1)	0.14
- Cancer	24 (13)	13 (10.9)	11 (16.7)	0.27
- Dementia	9 (4.9)	6 (5.0)	3 (4.5)	0.88
aPLs autoantibodies				
- LA			39 (59.1)	
- aCL IgG			22 (33.3)	
- aCL IgM			6 (9.1)	
- aB2GPI IgG			5 (7.6)	
- aB2GPI IgM			10 (15.2)	
02 > 6 L/min	58 (31.4)	33 (27.7)	25 (37.9)	0.15
ICU transfer	36/167 (21.6)	18/112 (16.1)	18/55 (32.7)	**0.014**
Thrombosis	10 (5.4)	4 (3.4)	6 (9.1)	0.17
Mechanical ventilation	7/167 (4.2)	5/112 (4.5)	2/55 (4.0)	0.69
Death	16 (8.6)	11 (9.2)	5 (7.6)	0.67

Abbreviations: aB2GPI, anti-beta-2 glycoprotein I; aCL, anticardiolipin; aPL, antiphospholipid; ICU, intensive care unit; LA, lupus anticoagulant; SD, standard deviation.
[a]Bold indicates statistical significance.

This might be due to the thromboprophylaxis given after discharge.

Our results support the hypothesis that severe forms of COVID-19 induce an autoimmune mechanism. Although reports are consistent with this hypothesis,[15] one caution of our study is that we are unaware of the aPL status before COVID-19 infection. However, aPLs are very rarely seen in the general population,[14] and most of our patients had no history of previous thrombosis. Moreover, the pathophysiological hypothesis underlying thrombosis related to aPLs proposed the involvement of two events[16]: a "first hit," consisting of the presence of aPLs, and another one associated with a "second hit" such as vascular damage or proinflammatory environment; COVID-19 meets the conditions of this second hit.

aPLs have also recently been identified as important link between thrombosis and inflammation. A new described mechanism showed that aCL recognizes a cell surface complex composed of lysophosphatidic acid and endothelial protein C receptor, thus activating toll-like receptors 7 and 8 (TLR-7 and TLR-8) and type I IFN signaling.[17] Thus, one can speculate that autoimmunity in COVID-19, in particular aPLs, could exacerbate inflammatory pathways and worsen the disease.

As a limitation, we acknowledge that the study was performed over a year ago. Although thromboprophylaxis management did not change, we cannot assess whether the change in COVID variant (mainly delta variant during the study) over time influenced the risk of developing APS. The patients with aPL had a reduced length of hospitalization, which is counterintuitive. Due to a higher incidence of

Table 2 Characteristics of the followed-up cohort at 12 weeks

Characteristics	Persistent aPL ($n = 13$)	Transient aPL ($n = 32$)	No aPL or transient aPL ($n = 151$)	*p*-Value
Age (mean ± SD)	68.3 ± 17	63.1 ± 13.6	63.5 ± 16.2	NS
Gender (female)	9 (69.2)	10 (31.2)	52 (34.4)	<0.05[a,b]
Comorbidities				
- Obesity	2 (15.4)	11 (34.4)	29/141 (20.6)	NS
- Pregnancy	0 (0)	1 (3.1)	2 (1.3)	NS
- Diabetes	0 (0)	9 (28.1)	42 (27.8)	<0.05[a,b]
- Hypertension	5 (38.5)	14 (43.8)	63 (41.7)	NS
- Chronic lung disease	3 (23.1)	3 (9.4)	20 (13.2)	NS
- Dyslipidemia	3 (23.1)	6 (18.8)	28 (18.5)	NS
- Cardiovascular disease	4 (30.8)	6 (18.8)	14 (9.3)	0.039[b]
- History of thromboembolic venous disease	1 (7.7)	1 (3.1)	2 (1.3)	NS
- Autoimmune disease	2 (15.4)	2 (6.3)	3 (2.0)	NS
- Chronic kidney failure	0 (0)	4 (12.5)	11 (7.3)	NS
- Immunosuppression	3 (23.1)	4 (12.5)	11 (7.3)	NS
- Cancer	3 (23.1)	5 (15.7)	18 (11.9)	NS
- Dementia	1 (7.7)	0 (0)	6 (4.0)	NS
Corticosteroid therapy	9 (69.2)	20 (62.5)		NS
02 > 6 L/min	5 (38.5)	7 (21.9)	40 (26.5)	NS
ICU transfer	3/10 (30.0)	7/28 (25.0)	25/140 (17.9)	NS
Thrombosis	3 (23.1)	2 (6.3)	6 (4.0)	0.025[b]
Mechanical ventilation	0 (0)	1/28 (3.6)	6/140 (4.3)	NS

Abbreviations: aB2GPI, anti-beta 2 glycoprotein I; aCL, anticardiolipin; aPL, antiphospholipid; ICU, intensive care unit.; LA, lupus anticoagulant; NS, nonsignificant; SD, standard deviation.
[a]Comparison between "persistent aPL" and "transient aPL."
[b]Comparison between "persistent aPL" and "no aPL or transient aPL."

thrombosis (stroke, acute coronary syndrome) or to a higher severity of the patients, more aPL patients were transferred to rehabilitation centers. When transferred to rehabilitation centers, they could have been discharged earlier than patients who were sent home because they were being monitored in the rehabilitation center.

In conclusion, APS remains a rare autoimmune disease but, its incidence is increased in COVID-19—particularly in patients who have experienced a thrombosis. Follow-up of aPLs should be recommended. Additional studies are needed to analyze how such patients could be better fitted into a thromboprophylaxis therapy.

Conflict of Interest
None declared.

References

1 WHO Coronavirus (COVID-19) Dashboard. Accessed April 25, 2021 at: https://covid19.who.int
2 Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 2020;130(05):2620–2629
3 Novelli L, Motta F, De Santis M, Ansari AA, Gershwin ME, Selmi C. The JANUS of chronic inflammatory and autoimmune diseases onset during COVID-19 - a systematic review of the literature. J Autoimmun 2021;117:102592
4 Taha M, Samavati L. Antiphospholipid antibodies in COVID-19: a meta-analysis and systematic review. RMD Open 2021;7(02): e001580
5 Favaloro EJ, Henry BM, Lippi G. Is lupus anticoagulant a significant feature of COVID-19? A critical appraisal of the literature. Semin Thromb Hemost 2022;48(01):55–71
6 Favaloro EJ, Henry BM, Lippi G. COVID-19 and antiphospholipid antibodies: Time for a reality check? Semin Thromb Hemost 2022;48(01):72–92
7 Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4 (02):295–306
8 Devreese KMJ, de Groot PG, de Laat B, et al. Guidance from the Scientific and Standardization Committee for lupus anticoagulant/antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis: Update of the guidelines for lupus anticoagulant detection and interpretation. J Thromb Haemost 2020;18(11):2828–2839
9 Bertin D, Brodovitch A, Beziane A, et al. Anticardiolipin IgG autoantibody level is an independent risk factor for COVID-19 severity. Arthritis Rheumatol 2020;72(11):1953–1955

10 Devreese KMJ, Linskens EA, Benoit D, Peperstraete H. Antiphospholipid antibodies in patients with COVID-19: a relevant observation? J Thromb Haemost 2020;18(09):2191–2201
11 Vollmer O, Tacquard C, Dieudonné Y, et al. Follow-up of COVID-19 patients: LA is transient but other aPLs are persistent. Autoimmun Rev 2021;20(06):102822
12 Gil-Etayo FJ, Garcinuño S, Lalueza A, et al. Anti-phospholipid antibodies and COVID-19 thrombosis: a co-star, not a supporting actor. Biomedicines 2021;9(08):899
13 Susen S, Tacquard CA, Godon A, et al. Traitement anticoagulant pour la prevention du risque thrombotique chez un patient hospitalise avec COVID-19 et surveillance de l’hemostase propositions du GIHP et du GFHT. Accessed September 9, 2022 at: https://sfar.org/download/traitement-anticoagulant-pour-la-prevention-du-risque-thrombotique-chez-un-patient-hospitalise-avec-covid-19-et-surveillance-de-lhemostase/
14 Duarte-García A, Pham MM, Crowson CS, et al. The epidemiology of antiphospholipid syndrome: a population-based study. Arthritis Rheumatol 2019;71(09):1545–1552
15 Rodríguez Y, Novelli L, Rojas M, et al. Autoinflammatory and autoimmune conditions at the crossroad of COVID-19. J Autoimmun 2020;114:102506
16 Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol 2011;7(06):330–339
17 Müller-Calleja N, Hollerbach A, Royce J, et al. Lipid presentation by the protein C receptor links coagulation with autoimmunity. Science 2021;371(6534):eabc0956