**Case Report**

# Dissecting the Thrombotic Events in the Presence of Antiphospholipid Antibodies in COVID-19 Spanish Patients

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### Abstract

Several thrombotic complications in patients affected with SARS-CoV-2 have been reported.

Single-centre pilot study aimed to analyse the coexistence of antiphospholipid antibodies and thrombotic events on SARS-CoV-2 infected patients.

Antiphospholipid antibodies were measured by solid phase enzyme immunoassay. Clinical data were collected from electronic history and clinical records.

Over 25 patients studied we report four cases of COVID-19 patients who presented circulating antiphospholipid antibodies and arterial or venous thrombotic events. No patient had a previous history of thrombosis. Two cases presented with pulmonary embolism, one with pulmonary embolism and pulmonary infarction and one with a stroke. All of them showed positive anti-cardiolipin antibodies. One patient died and three were discharged.

The presence of antiphospholipid antibodies in these patients might represent an epiphenomenon secondary to the immune stimulation by the virus. It could be reasonable to consider measuring antiphospholipid antibodies as part of the study of a thrombotic event in COVID-19 patients to better understand the impact of these antibodies in the development of thrombotic events and we highlight the value of a periodic determination to define the need of long-term anticoagulant therapy.

**Keywords:** Antiphospholipid antibodies, COVID-19, thrombosis

A novel coronavirus disease(COVID-19), caused by SARS-Cov-2 virus was reported at the end of 2019 and spread globally, affecting nearly all countries in the world.[1] Recently, several studies have reported thrombotic complications in patients affected with SARS-CoV-2, including deep vein thrombosis (DVT), large vessel stroke, pulmonary embolism (PE), coronary thrombosis, and systemic arterial and venous thromboembolism.[2–5] While critical illness is known to cause a hypercoagulable state due to immobilization, mechanical ventilation, central venous access devices, and nutritional deficiencies, COVID-19 itself appears to cause a hypercoagulable state through a bi-directional mechanism between inflammation and thrombosis.[6] Antiphospholipid syndrome (APS)is a systemic disorder

characterized by vascular thrombosis, recurrent miscarriage and the presence of antiphospholipid (aPL) antibodies.[7] Secondary cases of APS due to viral infections have been reported.[8,9] An entangled and interactive relationship between the viral infection and the host immune system mediate diverse aPL antibodies responses and related manifestations.[10] Cavalli et al. have hypothesized that there is a potential contribution of SARS-CoV-2 to induce a secondary APS in at least some cases of thrombotic events during the infection.[11]

We describe four patients infected with SARS-CoV-2 who presented with thrombotic events and circulating aPL antibodies.

## Methods

We study 25 patients who developed any thrombotic event (TE) during the admission for COVID-19. TE considered were: DVT, PE and stroke. TE were confirmed by duplex ultrasound in case of DVT, computed tomography (CT) in case of PE and for the stroke CT and magnetic resonance imaging. SARS-CoV-2 infection was established by real-time reverse transcriptase–polymerase chain reaction (PCR)of nasopharyngeal swabs.

Clinical data were prospectively collected during patient admission. We reviewed demographic information, medical history, comorbidities, and other laboratory data. We measured aPL in serum samples of the 25 patients, these samples were obtained for diagnostic purposes when the thrombotic event was suspected. IgG, IgM and IgA anti-cardiolipin antibodies (ACA) and anti-β2 glycoprotein I antibodies (B2GPI) were determined by solid phase enzyme immunoassay, ELISA (AESKULISA, AESKU DIAGNOSTICS). Lupus anticoagulant could not be determined because some patients started heparin very close to the TE. HLA Class I typing (locus A, B and C) was performed using the Sequence Specific Oligonucleotide reverse (SSO) technique through Luminexx MAP® technology. We employed the kits RSSOW1A, RSSOW1B and RSSOW1C (One lambda inc), which allows resolution at the level of Common and Well-Documented (CWD) alleles in a Flexmap 3D Luminex system. This study was approved by Ethical Committee of Ramón y Cajal University Hospital without the need to provide written informed consent due to the pandemic situation (Approval number 211-20).

## Results

Over 25 patients evaluated, we describe four cases with positive titers of aPL antibodies, TE and confirmed SARS-Cov-2 infection. Demographic data of patients are summarized in Table 1. All four patients were males; the mean age of patients was 51 years (range 35-62 years). Case1p-

resented with intermediate-low risk PE, case 2presented a pulmonary infarction secondary to a high-risk pulmonary embolism, case 3 presented with ischemic middle cerebral artery stroke (Fig. 1a) and case 4 showed a high-risk pulmonary embolism with hemodynamic instability (Fig. 1b). Three patients developed bilateral pneumonia and one presented with mild symptoms (cough, myalgia, and chest pain). Case 2, 3 and 4 needed intensive care unit (ICU) admission while the case 1 was admitted at medical ward. The mean time from disease onset to thrombotic event was 32 days (range, 6-90 days).All patients received anticoagulant therapy for the thrombotic events; three of them with low molecular weight heparin (LMWH) and one received unfractionated heparin. Cases 2 and 4 had antithrombotic prophylaxis during the admission before the thrombotic event. No patient receives antiplatelet or anticoagulant chronically prior to admission.

During TE, all patients had increased levels of D-dimer (mean 28468), ferritin (mean 3553) and fibrinogen (mean 642); about complement determination, 2 cases had high levels of C3 and one patient presented also with elevated level of C4. All four patients showed positive titers of ACA; case 1 and 3 had IgG ACA, case 2 showed IgA ACA and case 4 presented IgM ACA. No patient had anti B2GPI antibodies. Only in case 1 we can confirm the persistence of and IgG ACA 2 months after the discharge with lower levels (42.2 GPL/mL) but above the range of positivity, case 2 and 3 do not belong to this hospital area, and case 4 has died.

## Discussion

COVID-19 is a good example of how a viral infection can lead to a severe systemic inflammation and a prothrombotic state.[12] In this work, we have illustrated serious thrombotic events that some patients infected by SARS-Cov-2 have developed and the proposal of a possible role of aPL antibodies to trigger a secondary APS, which certainly raises the idea that measuring aPL antibodies in COVID-19 may be worth.

The first cases of coagulopathy and aPL antibodies in patients with COVID-19 were published in April 2020, where the authors reported these antibodies as an observation that hardlycan explain TE in critical patients.[13] Over the last months, several studies have reported the presence of aPL antibodies in patients infected with SARS-CoV-2.[12,14,15] By contrast to Pineton et al. who reach an exceptionally high frequency of aPL antibodies,[12] in our series only 16% of the patients (4/25) showed positive aPL antibodies; our results are similar to other that shows a low prevalence of aPL antibodies in patients with TE.[15,16] Devreese et al. found transient aPL not clearly related to TE[14] while Zuo et al.[17] suggest a potential pathogenic role of aPL antibodies in a

**Table 1.** clinical features and antiphospolipid antibodies' profile in four patients with TE and COVID-19 infection.

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| **Demographical data** | | | | |
| Age; gender | 35; male | 62; male | 54; male | 55; male |
| Medical history | None | Type 2 diabetes, overweight, ex-smoker | Non-obstructive HC | OSAHS |
| **Features related to thrombotic events** | | | | |
| Thrombotic event | PE | PE + pulmonary infarction | Stroke | PE |
| Days from disease onset to TE | 24 | 28 | N/A (asymtomatic patient) | 30 |
| Anticoagulation before TE | No | LMWH 60mg/12h | No | LMWH 40mg/24h |
| Days of hospital stay | 8 | 90 | 6 | 25 |
| ICU | No | Yes | Yes | Yes |
| Antithrombotic therapy | LMWH 80mg/12h | LMWH | Fibrinolysis, Acetylsalicylic acid, Thrombectomy, LMWH 60mg/24h | IV UFH |
| Outcome | Discharge | Discharge | Discharge | Exitus |
| **Laboratory findings** | | | | |
| Complement (mg/dL) | C3 139 | C3 215 | C3 695 | C3 349 |
| | C4 23.2 | C4 3.4 | C4 85.5 | C4 140 |
| | N/A | CH50 < 7.49 | N/A | N/A |
| Fibrinogen (mg/dL) | 669,7 | 740 | 317,8 | 740 |
| D-dimer (ng/mL) | 2447 | 24814 | 1611 | 85000 |
| Serum ferritin (ng/mL) | 1222 | 9963 | 187 | 2741 |
| Anti-cardiolipin antibodies* | IgG 70.4 GPL/µL | IgG 5.6 GPL/µL | IgG 20.3 GPL/µL | IgG 2.76 GPL/µL |
| | IgM 5.7 MPL/µL | IgM 2.48 MPL/µL | IgM 2.63 MPL/µL | IgM 18.6 MPL/µL |
| | IgA 15.2 APL/µL | IgA 20.4 APL/µL | IgA 2.76 APL/µL | IgA 0.76 APL/µL |
| Anti-beta2-glycoprotein 1 antibodies * | IgG 8.22 U/µL | IgG 2.08 U/µL | IgG 4.57 U/µL | IgG 1.54 U/µL |
| | IgM 5.89 U/µL | IgM 1.66 U/µL | IgM 3.32 U/µL | IgM 3.47 U/µL |
| | IgA 1.76 U/µL | IgA 9.52 U/µL | IgA 0.46 U/µL | IgA 0.35 U/µL |
| HLA | DRB1*03 DRB1*16 DRB3+ DRB5+ DQB1*05 DQB1*02 | DRB1*03 DRB1*04 DRB3+ DRB4+ DQB1*02 QB1*03:02 | N/A | DRB1*15 DRB5+ DQB1*06 |

HC: hypertrophic cardiomyopathy, hypertrroLMWH: low molecular weight heparin, N/A: not available, OSAHS: Apnea-hypopnea syndrome, PE: pulmonary embolism, UFH: Unfractionated Heparin: *Cut-off point: negative <12, weak-positive 12-18, positive >18.
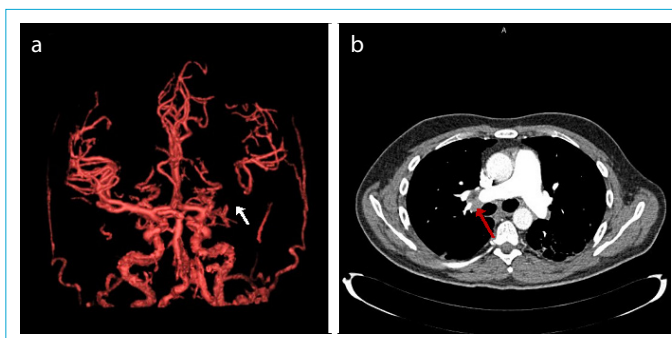


**Figure 1. (a)** 3D reconstruction of supra-aortic trunk angiography and circle of Willis, the arrow shows a repletion defect in left middle cerebral artery. **(b)** Torax- CT: the arrow shows a filling defect in the right pulmonary artery.

significant percentage of patients with COVID-19. Borghi et al. found by ELISA a higher frequency of anti-β2-GPI over ACA, differing to our results that show ACA positive in all four cases.[15] About the clinical features of the TE, we found a higher frequency of PE rather than other thrombotic complications, these results are in line with other previously published.[18]

Although the specific factors resulting in the induction of aPL and the associated thrombotic event are still unknown, we analyze the immunological pathways that could rationalize the presence of aPL antibodies and TE in patients with COVID-19. SARS-Cov-2 infection. It may represent a clinical epiphenomenon of a viral-induced secondary APS.[11] For years, the relationship of infectious diseases in the development of APS has been known.[10] It has been reported that infections may trigger the production of pathogenic aPL in certain predisposed individuals.[8] One proposal is that aPL antibodies may emerge by a molecular mimicry mechanism due to the similarity of certain peptides of viruses and bacteria with β2-GPI.[19,20] The presence of these

autoantibodies can promote, on the one hand, a state of hypercoagulability, and on the other, a systemic inflammatory response by the activation of the Toll-Like receptor 4 (TLR-4)[21] that can cause the massive production IL-1, IL-5, IL-6, IFN-γ and TNF-α.[19] SARS-Cov-2 upregulates the release of proinflammatory cytokines by the innate immune system leading to a systemic inflammatory response,[11] followed by endothelial alterations that induce a procoagulant state.[19] This state is mainly mediated by the increased synthesis of tissue factor and thromboxane A2 as well as an activation of the complement cascade that might close the loop and provoke thrombosis.[11] Most of the patients studied also had elevated levels of ferritin. There is evidence that ferritin levels not only reflect an acute phase response, but rather they have an active role in the inflammation process.[19] Ferritin acts as a signalling molecule and mediator of immune processes, probably through binding to its T-cell immunoglobulin and mucin domain 2 (TIM-2) receptor[22] and also negatively regulates cells involved in Th2 immune reaction, influencing immune tolerance and autoimmunity.[19] At the same time, macrophage-mediated ferritin secretion has been correlated with thrombosis through the induction of inflammatory molecules such as ICAM1 (intercellular adhesion molecule 1) which can contribute to the development of a cytokine storm and the activation of endothelial cells.[23,24] Severe cases of SARS-CoV-2 have shown extreme levels of D-dimer elevation, thrombocytopenia and coagulopathy regulated by pro-inflammatory soluble mediators which could be an early indicator for hospitalization, disease severity, prognosis and may help physicians take timely clinical decisions.[25]

Additionally, several studies have revealed the existence of a genetic predisposition for the development of APS, specially by its association with various human leukocyte antigen (HLA) alleles.[26,27] Sebastiani et al. reported the association of HLA DR4, DR7, DRw53, and DQB1*0302 with the production of aPL antibodies.[27] In this regard, one case presented in our work showed a risk genotype (Table 1).

## Conclusion

The presence of aPL antibodiesmight represent an epiphenomenon secondary to the immune stimulation by the SARS-CoV-2; since the role of aPL antibodies in the development of thrombosis is still controversial. If some or most cases of TE in COVID 19 represent a secondary APS, this could raise the idea that aPL antibodies can be measured as part of the study of a thrombotic event. In positive patients, a second determination, once the infection has been resolved, would be recommended because, if aPL antibodies persist positive, they could determine a long-term anticoagulation. Further studies to demonstrate a pathogenic

correlation between thrombosis, SARS-Cov-2 and the presence of aPL antibodies are necessary.

## References

1.  Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.

2.  Machhi J, Herskovitz J, Senan AM, Dutta D, Nath B, Oleynikov MD, et al. The natural history, pathobiology, and clinical manifestations of SARS-CoV-2 infections. J Neuroimmune Pharmacol 2020;15:359–86.

3.  Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res 2020;191:145–7.

4.  Gomez-Arbelaez D, Ibarra-Sanchez G, Garcia-Gutierrez A, Comanges-Yeboles A, Ansuategui-Vicente M, Gonzalez-Fajardo JA. COVID-19-Related Aortic Thrombosis: A Report of Four Cases. Ann Vasc Surg 2020;67:10–3.

5.  Shams A, Ata F, Mushtaq K, Munir W, Yousaf Z. Coronary thrombosis in a young male with COVID-19. IDCases 2020;21:e00923.

6.  Abou-Ismail MY, Diamond A, Kapoor S, Arafah Y, Nayak L. The hypercoagulable state in COVID-19: Incidence, pathophysiology, and management. Thromb Res 2020;194:101–15.

7.  Rodziewicz M, D'Cruz DP. An update on the management of antiphospholipid syndrome. Ther Adv Musculoskelet Dis 2020;12:1759720X20910855.

8.  Cervera R, Asherson RA, Acevedo ML, Gómez-Puerta JA, Espinosa G, De La Red G, et al. Antiphospholipid syndrome associated with infections: clinical and microbiological characteristics of 100 patients. Ann Rheum Dis 2004;63:1312–7.

9.  Plummer MP, Young AMH, O'Leary R, Damian MS, Lavinio A. Probable Catastrophic Antiphospholipid Syndrome with intracerebral hemorrhage secondary to Epstein-Barr Viral infection. Neurocrit Care 2018;28:127–32.

10. Martirosyan A, Aminov R, Manukyan G. Environmental Triggers of Autoreactive Responses: Induction of Antiphospholipid Antibody Formation. Front Immunol 2019;10:1609.

11. Cavalli E, Bramanti A, Ciurleo R, Tchorbanov AI, Giordano A, Fagone P, et al. Entangling COVID-19 associated thrombosis into a secondary antiphospholipid antibody syndrome: Diagnostic and therapeutic perspectives (Review). Int J Mol Med

2020;46:903–12.

12. Pineton de Chambrun M, Frere C, Miyara M, Amoura Z, Martin-Toutain I, Mathian A, et al. High frequency of antiphospholipid antibodies in critically ill COVID-19 patients: a link with hypercoagulability? J Intern Med 2021;289:422–4.

13. Zhang Y, Xiao M, Zhang S, Xia P, Cao W, Jiang W, et al. Coagulopathy and antiphospholipid antibodies in patients with Covid-19. N Engl J Med 2020;382:e38.

14. Devreese KMJ, Linskens EA, Benoit D, Peperstraete H. Antiphospholipid antibodies in patients with COVID-19: A relevant observation? J Thromb Haemost 2020;18:2191–201.

15. Borghi MO, Beltagy A, Garrafa E, Curreli D, Cecchini G, Bodio C, et al. Anti-phospholipid antibodies in COVID-19 are different from those detectable in the Anti-Phospholipid Syndrome. Front Immunol 2020;11:584241.

16. Galeano-Valle F, Oblitas CM, Ferreiro-Mazón MM, Alonso-Muñoz J, Del Toro-Cervera J, di Natale M, et al. Antiphospholipid antibodies are not elevated in patients with severe COVID-19 pneumonia and venous thromboembolism. Thromb Res 2020;192:113–5.

17. Zuo Y, Estes SK, Ali RA, Gandhi AA, Yalavarthi S, Shi H, et al. Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. Sci Transl Med 2020;12:eabd3876.

18. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al; CRICS TRIGGERSEP Group (Clinical Research in Intensive Care and Sepsis Trial Group for Global Evaluation and Research in Sepsis). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med 2020;46:1089–98.

19. Garcia-Carrasco M, Mendoza-Pinto C, Macias-Diaz S, Vazquez de Lara F, Etchegaray-Morales I, Galvez-Romero JL, et al. The role of infectious diseases in the catastrophic antiphospholipid syndrome. Autoimmun Rev 2015;14:1066–71.

20. Asherson RA. The catastrophic antiphospholipid (Asherson's) syndrome. Autoimmun Rev 2006;6:64–7.

21. Raschi E, Borghi MO, Grossi C, Broggini V, Pierangeli S, Meroni PL. Toll-like receptors: another player in the pathogenesis of the anti-phospholipid syndrome. Lupus 2008;17:937–42.

22. Han J, Seaman WE, Di X, Wang W, Willingham M, Torti FM, et al. Iron uptake mediated by binding of H-ferritin to the TIM-2 receptor in mouse cells. PLoS One 2011;6:e23800.

23. Cronin SJF, Woolf CJ, Weiss G, Penninger JM. The Role of Iron Regulation in Immunometabolism and Immune-Related Disease. Front Mol Biosci 2019;6:116.

24. Agmon-Levin N, Rosário C, Katz BS, Zandman-Goddard G, Meroni P, Cervera R, et al. Ferritin in the antiphospholipid syndrome and its catastrophic variant (cAPS). Lupus 2013;22:1327–35.

25. Kaur S, Bansal R, Kollimuttathuillam S, Gowda AM, Singh B, Mehta D, et al. The looming storm: Blood and cytokines in COVID-19. Blood Rev 2021;46:100743.

26. Cervera R, Rodríguez-Pintó I, Colafrancesco S, Conti F, Valesini G, Rosário C, et al. 14th International Congress on Antiphospholipid Antibodies Task Force Report on Catastrophic Antiphospholipid Syndrome. Autoimmun Rev 2014;13:699–707.

27. Domenico Sebastiani G, Minisola G, Galeazzi M. HLA class II alleles and genetic predisposition to the antiphospholipid syndrome. Autoimmun Rev 2003;2:387–94.