Prevalence, specificity and clinical association of anti-phospholipid antibodies in COVID-19 patients: are the antibodies really guilty?

Maria Orietta Borghi 1,2, Asmaa Beltagy1,3, Emirena Garrafa4,5, Daniele Curreli 1, Germana Cecchini 6, Caterina Bodio 1, Claudia Grossi 1, Simonetta Blengino 7, Angela Tincani 8, Franco Franceschini 8, Laura Andreoli 8, Maria Grazia Lazzaroni 8, Silvia Piantoni 8, Stefania Masneri 8, Francesca Crisafulli 8, Duilio Brugnoni 5, Maria Lorenza Muiesan 9, Massimo Salvetti 9, Gianfranco Parati 7, Erminio Torresani 6, Michael Mahler 10, Francesca Heilbron 7, Francesca Pregnolato 1, Martino Pengo 7, Francesco Tedesco 1, Nicola Pozzi 11,*, Pier Luigi Meroni 1,*

1Istituto Auxologico Italiano, IRCCS, Immunorheumatology Research Laboratory, Milan, Italy.
2Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy.
3Rheumatology and Clinical Immunology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt.
4Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.
5Department of Laboratory Diagnostics, ASST Spedali Civili, Brescia, Italy.
6Istituto Auxologico Italiano, IRCCS, Department of Chemical Chemistry, Milan, Italy.
7Istituto Auxologico Italiano, IRCCS, Department of Cardiovascular, Neural and Metabolic Sciences, San Luca Hospital, Milan, Italy.
8Rheumatology and Clinical Immunology Unit, Department of Clinical and Experimental Sciences, ASST Spedali Civili and University of Brescia, Brescia, Italy.
9UOC 2° Medicina, Department of Clinical and Experimental Sciences, ASST Spedali Civili and University of Brescia, Brescia, Italy.
10Inova Diagnostics, Inc., San Diego, CA, USA.
11Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis, USA.
*Corresponding authors:*

Pier Luigi Meroni, e-mail: pierluigi.meroni@unimi.it

Nicola Pozzi, e-mail: nicola.pozzi@health.slu.edu

**Running Head:** Anti-phospholipid antibodies and COVID-19

**Key words:** anti-phospholipid antibodies, β₂-glycoprotein I, prothrombin, COVID-19, autoimmunity, antiphospholipid syndrome, thrombosis.

**Summary points**

- A relationship between COVID-19 coagulopathy and aPL has been proposed, but results are controversial.
- aCL/anti-β₂GPI/aPS/PT and anti-D1/D4-5 β₂GPI antibodies were tested in sera from severe COVID-19.
- Low titer aPL were found in a small proportion of patients and there was no association with thrombosis.
- aPL in COVID-19 are mainly directed against β₂GPI but display an epitope specificity different from APS.
Abstract

Background. Critically ill patients with coronavirus disease 2019 (COVID-19) have a profound hypercoagulable state and often develop coagulopathy which leads to organ failure and death. Because of a prolonged activated partial-thromboplastin time (aPTT), a relationship with anti-phospholipid antibodies (aPL) has been proposed, but results are controversial. Functional assays for aPL (i.e., lupus anticoagulant) can be influenced by concomitant anticoagulation and/or high levels of C reactive protein. The presence of anti-cardiolipin (aCL), anti-beta2-glycoprotein I (anti-β2GPI) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies was not investigated systematically. Epitope specificity of anti-β2GPI antibodies was not reported.

Aim. To evaluate the prevalence and the clinical association of aPL in a large cohort of COVID-19 patients, and to characterize the epitope specificity of anti-β2GPI antibodies.

Methods. ELISA and chemiluminescence assays were used to test 122 sera of patients suffering from severe COVID-19. Of them 16 displayed major thrombotic events.

Results. Anti-β2GPI IgG/IgA/IgM were the most frequent in 15.6/6.6/9.0% of patients, while aCL IgG/IgM were detected in 5.7/6.6% by ELISA. Comparable values were found by chemiluminescence. aPS/PT IgG/IgM were detectable in 2.5 and 9.8% by ELISA. No association between thrombosis and aPL was found. Reactivity against domain 1 and 4-5 of β2GPI was limited to 3/58 (5.2%) tested sera for each domain and did not correlate with aCL/anti-β2GPI nor with thrombosis.

Conclusion. aPL show a low prevalence in COVID-19 patients and are not associated with major thrombotic events. aPL in COVID-19 patients are mainly directed against β2GPI but display an epitope specificity different from antibodies in antiphospholipid syndrome.
INTRODUCTION

Critically ill patients with coronavirus disease 2019 (COVID-19) have a profound hypercoagulable state and often develop thrombosis in veins, arteries and in the microcirculation [1]. As such, these patients may experience organ failure and eventually die [2]. Recent analysis of coagulation screening parameters were instrumental at identifying several abnormalities in these patients, including prominent elevation of fibrin/fibrinogen degradation products (i.e., D-dimer) and a prolonged activated partial-thromboplastin time (aPTT). While high levels of D-dimer are consistent with sustained activation of the clotting and fibrinolytic cascades, the combination of prolonged aPTT and both arterial and venous thrombosis was, however, surprising, and it is reminiscent of a clinical scenario known as antiphospholipid syndrome (APS) [3]. APS is a systemic autoimmune disorder characterized by venous and arterial thrombosis in the presence of anti-phospholipid antibodies (aPL) that can prolong phospholipid-dependent clotting time assays [3].

Looking at the causes for aPTT prolongation, recent studies have shown that lupus anticoagulant (LA) can be detected in a significant percentage of COVID-19 positive samples [4-6]. Since LA is often caused by aPL, these findings support the idea that aPL may play a role in COVID-19 [7]. However, it is important to point out that LA is a very sensitive assay and its outcome can be influenced by several factors, most notably heparin administration [8] and a profound inflammatory state characterized by high levels of C reactive protein (CRP) [9-11]. Both of them are present in COVID-19 patients [12].

Another method to detect aPL that is in principle insensitive to anticoagulation and other confounding agents relies on the detection and quantification of autoantibodies using solid phase assays [3]. Using this method, the presence of aPL was recently reported in a handful of case reports and a small cohort of patients [4-7]. While encouraging, this data is limited and its interpretation remains controversial, with some investigators proposing an important role of aPL in COVID-19 patients [7] while others suggesting a very poor correlation between aPL and thrombotic...
events [2]. A larger study, possibly multicenter, would be ideal to clarify these outstanding issues and draw conclusions regarding the prevalence, epitope specificity and clinical correlation between aPL and thrombotic manifestations in COVID-19 patients. To fill this gap in knowledge, we used a combination of ELISA and chemiluminescence assays to quantify the levels and identify the type of aPL present in 122 sera of patients suffering from severe or critical COVID-19. Our data indicates a low prevalence of aPL in COVID-19 patients. Yet, they are not associated with major thrombotic events. Importantly, aPL in COVID-19 patients are mainly reacting against β2-glycoprotein I (β2GPI) but display an epitope specificity different from antibodies found in APS patients.

**MATERIALS & METHODS**

**Patients**

A total of 122 patients were enrolled from two COVID-19 referral centers in Lombardia, which is one of the regions in Northern Italy heavily affected by the pandemic. Specifically, 58 patients were recruited in Milan and 64 patients were recruited in Brescia. All patients tested positive to SARS-CoV-2, as detected by RT-qPCR [12]. Mean age was 68.5 (± SD 16.4) years; 77 were men and 45 women. All 122 patients were classified as severe or critical COVID-19 according to Chen et al. [12]. No diagnosis of previous autoimmune diseases was made; six patients had a thrombotic event (3 arterial and 3 venous) in the past clinical history. Eighty-seven patients suffering from APS were also tested for aCL and anti-β2GPI IgG, IgM [13]. The study was approved by the Ethics Committees in Milan (Ethic Committee Istituto Auxologico Italiano 3-04-2020) and Brescia (ASST Spedali Civili NP4187).

**Detection of aPL**

Anti-cardiolipin (aCL) and anti-β2GPI IgG, IgA and IgM were detected by chemiluminescence immunoassay (CIA; Quanta Flash, Inova, San Diego, CA, US) and by a home-made ELISA as described elsewhere [13, 14]. Anti-β2GPI domain 1 IgG (anti-D1) were detected by CIA (Inova).
IgG anti-D4-5 were detected by a home-made ELISA, as described previously [13, 14]. Anti-phosphatidylserine/prothrombin (aPS/PT) IgG and IgM were detected by a commercial ELISA (Inova) as reported [15].

**Statistical analysis**

Data were analyzed using R version 3.4.0. Descriptive statistics was used to summarize data. Associations and differences between categorical or continuous variables were tested by using Fisher’s exact test and non-parametric Mann-Whitney test, respectively. A p-value <0.05 was considered statistically significant.

**RESULTS & DISCUSSION**

Eighty-three per cent of COVID-19 patients enrolled in our study displayed elevated D-dimer and elevated levels of C reactive protein (CRP). Prolonged aPTT (>30 sec) was found in 57.6% of COVID-19 patients while PT INR values were above the cut-off in 24.8% of the cases. Most of the patients (120 out of 122) were on anticoagulation with low molecular weight heparin (70% of the patients was on therapeutic and the remaining on prophylactic heparin dosage). Despite anticoagulation, we observed sixteen thrombotic events (13.1%), of which 8 were in veins and 8 in arteries. These statistics are in agreement with previous reports [2, 16-21] and document abnormal coagulation activities in our patient population.

In the APS field, testing for LA is not recommended by the official guidelines when patients are on heparin, since the presence of heparin, even if neutralized, may lead to false positive results [8]. Likewise, high levels CRP, such as those found in our cohort of patients, have been shown to prolong aPTT independently from the presence of aPL [9-11]. On these bases, the presence of aPL were researched using solid phase assays, and not LA. First, we investigated the presence of aCL and anti-β2GPI, two very established classification criteria diagnostic tools in APS [3]. Testing was independently performed at two different sites, Milan and Brescia, using standardized
methodologies harmonized between the two laboratories [22]. The prevalence of COVID-19 patients positive for IgG/IgA/IgM aCL and anti-β2GPI antibodies detected by ELISA and CIA is summarized in Table 1. The raw data of the ELISA assays are shown in Figure 1. We found IgG/IgM aCL in 5.7/6.6% of patients, whereas anti-β2GPI IgG/IgA/IgM were found in 15.6/6.6/9.0% of patients. Similar values were obtained for aCL antibodies using CIA assays (Table 1) [23], whereas a slightly lower sensitivity was obtained for anti-β2GPI antibodies. Taken together, our data shows a low prevalence of classification criteria aPL in COVID-19 patients. In this regard, our study confirms a recent study obtained with smaller cohorts of patients [4, 21]. Importantly, our data also shows that aPL are slightly more reactive towards β2GPI-coated plates as compared to CL-coated ones and that, regardless of the nature of aPL, there is no association between aPL positivity and thrombotic events (p = 1).

A striking difference between the autoantibody profile found in COVID-19 patients as compared to the one found in APS concerned the titers of aPL. Medium/low aPL titers were consistently found in the patients with COVID-19. By contrast, medium/high titers are usually found in APS patients (Figure 1). This difference suggests that aPL found in COVID-19 may be different from aPL found in APS and led us to further investigate the epitope specificity of anti-β2GPI antibodies. We focused on autoantibodies directed against the N-terminal domain 1 (anti-D1) or the C-terminal domains 4-5 (anti-D4-5) of the molecule [14] (Figure 2A). This is because anti-D1 antibodies are associated with an increased risk of thrombosis and pregnancy complications in APS patients [13, 14, 24]. By contrast, anti D4-5 antibodies, are associated neither with vascular nor obstetric APS manifestations [13, 25]. Furthermore, anti D4-5 antibodies are also reported at high levels in the so called asymptomatic aPL carriers and are frequently found in non-APS (e.g. patients with leprosy, atopic dermatitis, atherosclerosis and in children born to mothers with systemic autoimmune diseases) [25]. Figure 2B shows that three out of 58 samples reacted with D1, while in Figure 2C, three samples tested positive for D4-5. None of the sera was positive for both domains and all displayed a weak reactivity. Although the number of the investigated sera is relatively small, this finding is
quite different from the results found in APS in which almost all the sera positive for the whole β₂GPI molecule also reacted with domain D1 at high titer [13, 24]. Furthermore, at variance with APS patients, none of the anti-D1 positive patients displayed thrombotic events [24].

Approximately fifty-seven per cent of COVID-19 patients has prolonged aPTT. Yet, only a small proportion of COVID-19 patients carry aCL and anti-β₂GPI antibodies. This suggests that other factors must be responsible for the prolonged aPTT phenomenon. Based on current literature, aPS/PT can be associated with a prolonged aPTT and with the presence of LA [15]. We found fifteen out of 122 sera positive for aPS/PT (12.3%), mostly of the IgM isotype (12 out 15) and at a low titer (Figure 3). There was no association between prolonged aPTT and the presence of aPS/PT antibodies nor with thrombotic events in our COVID-19 cohort. This indicates that aPS/PT are not responsible for the prolongation of aPTT nor are a predictor of adverse clinical outcomes. Furthermore, in contrast to what we would have expected in APS [26], we found no associations between the presence of aPS/PT, aCL and anti-β₂GPI antibodies. This data is in line with the unusual epitope specificity of anti-β₂GPI antibodies documented in Figure 2, supporting the hypothesis that aPL found in COVID-19 patients are different from aPL found in APS patients. Whether COVID-19 aPL are similar to the one found in other infectious diseases such HCV, HBV and HIV [27] remains to be determined.

In conclusion, this study documents a low prevalence of aPL in COVID-19 patients and found no association between aPL positivity and major thrombotic events. This leads to the conclusion that aPL are not the main cause of prolonged aPTT nor can explain the pathogenesis of venous and arterial thrombosis in COVID-19 patients. While testing for aPL in COVID-19 patients cannot be recommended at this time, it is important to keep in mind that COVID-19 patients suffer of an acute form of systemic inflammation with complement activation [28], which may be responsible for endothelial perturbation. In this context, since β₂GPI can accumulate on the activated endothelium at high density, even low titers of aPL may become pathogenic thus potentiating or even triggering thrombus formation, especially when anticoagulation is suspended. A comparable condition in
which low titers of aPL can cause substantial damage is seen in obstetric APS, where high levels of β2GPI can be found in the placenta [29]. Hence, while transitory aPL are likely to be clinically irrelevant in COVID-19 patients as in other infections [27], detection of persistent aPL (12 weeks apart from the first detection) may represent a true risk factor. Accordingly, aPL detection may offer a more robust risk stratification for thrombosis and could eventually justify post-discharge anticoagulant prophylaxis.

AUTHORSHIP
M.O. Borghi, M. Pengo, A. Tincani, F. Franceschini, F. Tedesco, N. Pozzi, P.L. Meroni designed the study; S. Blengino, G. Parati, F. Heilbron, M. Pengo, M.G. Lazzaroni, M.L. Muiesan, M. Salvetti collected clinical samples; E. Garrafa, D. Curreli, G. Cecchini, C. Bodio, C. Grossi, S. Piantoni, S. Masneri, F. Crisafulli, D. Brugnoni, E. Torresani, M. Mahler, L. Andreoli, performed research; M.O. Borghi, A. Beltagy, F. Pregnolato, F. Tedesco, N. Pozzi, P.L. Meroni analyzed data; M.O. Borghi, F. Tedesco, N. Pozzi, P.L. Meroni wrote the manuscript. All authors reviewed and approved the manuscript.

ACKNOWLEDGEMENT
The study was in part supported by IRCCS Istituto Auxologico Italiano - Ricerca Corrente 2019 (PL Meroni), a grant from the Italian Ministry of Foreign Affairs and International Cooperation (MAECI) for foreign citizens and Italian citizens living abroad (A. Beltagy) and a National Institutes of Health Research Grant HL150146 (N. Pozzi). The Authors would like to thank: Drs. N. Carabellese and G. Martini (Department of Laboratory Diagnostics; ASST Spedali Civili, Brescia, Italy) for their valuable collaboration; all the physicians of the COVID-19 Units of the IRCCS Istituto Auxologico Italiano (Milan) and the ASST Spedali Civili (Brescia).

CONFLICTS OF INTEREST
M. Mahler is an employee at Inova Diagnostics, Inc. All the other authors declared no conflict of interest.

REFERENCES

1. Carsana L, Sonzogni A, Nasr A, Rossi R, Pellegrinelli A, Zerbi P, Rech R, Colombo R, Antinori S, Corbellino M, Galli M, Catena E, Tosoni A, Gianatti A, Nebuloni M. Pulmonary post-mortem findings in a large series of COVID-19 cases from Northern Italy. *medRxiv*. 2020; 2020.04.19.20054262. 10.1101/2020.04.19.20054262.

2. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *Journal of Thrombosis and Haemostasis*. 2020; 18: 844-7. 10.1111/jth.14768.

3. Garcia D, Erkan D. Diagnosis and Management of the Antiphospholipid Syndrome. *New England Journal of Medicine*. 2018; 378: 2010-21. 10.1056/NEJMra1705454.

4. Harzallah I, Debliquis A, Drénou B. Lupus anticoagulant is frequent in patients with Covid-19. *Journal of Thrombosis and Haemostasis*. 2020; n/a. 10.1111/jth.14867.

5. Bowles L, Platton S, Yartey N, Dave M, Lee K, Hart DP, MacDonald V, Green L, Sivapalaratnam S, Pasi KJ, MacCallum P. Lupus Anticoagulant and Abnormal Coagulation Tests in Patients with Covid-19. *New England Journal of Medicine*. 2020. 10.1056/NEJMc2013656.

6. Pineton de Chambrun M, Frere C, Miyara M, et al. High Frequency of Antiphospholipid Antibodies in Critically-ill COVID-19 Patients: a Link with Hypercoagulability? [published online ahead of print, 2020 Jun 12]. *J Intern Med*. 2020;10.1111/joim.13126. doi:10.1111/joim.13126

7. Zhang Y, Xiao M, Zhang S, Xia P, Cao W, Jiang W, Chen H, Ding X, Zhao H, Zhang H, Wang C, Zhao J, Sun X, Tian R, Wu W, Wu D, Ma J, Chen Y, Zhang D, Xie J, Yan X, Zhou X, Liu Z, Wang J, Du B, Qin Y, Gao P, Qin X, Xu Y, Zhang W, Li T, Zhang F, Zhao Y, Li Y, Zhang S. Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19. *New England Journal of Medicine*. 2020; 382: e38. 10.1056/NEJMc2007575.
8. Martinuzzo ME, Barrera LH, D’Adamo MA, Otaso JC, Gimenez MI, Oyhamburu J. Frequent False-positive results of lupus anticoagulant tests in plasmas of patients receiving the new oral anticoagulants and enoxaparin. *International Journal of Laboratory Hematology*. 2014; 36: 144-50. 10.1111/ijlh.12138.

9. Schouwers SME, Delanghe JR, Devreese KMJ. Lupus Anticoagulant (LAC) testing in patients with inflammatory status: Does C-reactive protein interfere with LAC test results? *Thrombosis Research*. 2010; 125: 102-4. 10.1016/j.thromres.2009.09.001.

10. Ruinemans-Koerts J, Ahmed-Ousenkova YM, Kaasjager HAH, Hendriks-van Wijhe C, Hovens MMC. When to screen for lupus anticoagulant? Influence of testing during acute phase and consequences for clinical practise. *Lupus*. 2015; 24: 1233-5. 10.1177/0961203315583540.

11. Wenzel C, Stoiser B, Locker GJ, Laczika K, Quehenberger P, Kapiotis S, Frass M, Pabinger I, Knöbl P. Frequent development of lupus anticoagulants in critically ill patients treated under intensive care conditions. *Crit Care Med*. 2002; 30: 763-70. 10.1097/00003246-200204000-00007.

12. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang X, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. *The Journal of Clinical Investigation*. 2020; 130: 2620-9. 10.1172/JCI137244.

13. Chighizola CB, Pregnolato F, Andreoli L, Bodio C, Cesana L, Comerio C, Gerosa M, Grossi C, Kumar R, Lazzaroni MG, Mahler M, Mattia E, Nalli C, Norman GL, Raimondo MG, Ruffatti A, Tonello M, Trespidi L, Tincani A, Borghi MO, Meroni PL. Beyond thrombosis: Anti-β2GPI domain 1 antibodies identify late pregnancy morbidity in anti-phospholipid syndrome. *J Autoimmun*. 2018; 90: 76-83. 10.1016/j.jaut.2018.02.002.

14. Durigutto P, Grossi C, Borghi MO, Macor P, Pregnolato F, Raschi E, Myers MP, de Groot PG, Meroni PL, Tedesco F. New insight into antiphospholipid syndrome: antibodies to β2glycoprotein I-domain 5 fail to induce thrombi in rats. *Haematologica*. 2019; 104: 819-26. 10.3324/haematol.2018.198119.
15  Tincani A, Morozzi G, Afeltra A, Alessandri C, Allegri F, Bistoni O, Bizzaro N, Caccavo D, Galeazzi M, Gerli R, Giovannelli L, Longobardo G, Lotzniker M, Malacarne F, Migliorini P, Parodi A, Pregnolato F, Radice A, Riccieri V, Ruffelli M, Sinico RA, Tozzoli R, Villalta D, Marcolongo R, Meroni P. Antiprothrombin antibodies: a comparative analysis of homemade and commercial methods. A collaborative study by the Forum Interdisciplinare per la Ricerca nelle Malattie Autoimmuni (FIRMA). *Clinical and experimental rheumatology*. 2007; 25: 268-74.

16  Nahum J, Morichau-Beauchant T, Daviaud F, Echegut P, Fichet J, Maillet J-M, Thierry S. Venous Thrombosis Among Critically Ill Patients With Coronavirus Disease 2019 (COVID-19). *JAMA Network Open*. 2020; 3: e2010478-e. 10.1001/jamanetworkopen.2020.10478.

17  Zhang L, Feng X, Zhang D, Jiang C, Mei H, Wang J, Zhang C, Li H, Xia X, Kong S, Liao J, Jia H, Pang X, Song Y, Tian Y, Wang B, Wu C, Yuan H, Zhang Y, Li Y, Sun W, Zhang Y, Zhu S, Wang S, Xie Y, Ge S, Zhang L, Hu Y, Xie M. Deep Vein Thrombosis in Hospitalized Patients with Coronavirus Disease 2019 (COVID-19) in Wuhan, China: Prevalence, Risk Factors, and Outcome. *Circulation*. 0. 10.1161/CIRCULATIONAHA.120.046702.

18  Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, Kaptein FHJ, van Paassen J, Stals MAM, Huisman MV, Endeman H. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thrombosis Research*. 2020; 191: 145-7. https://doi.org/10.1016/j.thromres.2020.04.013.

19  Lodigiani C, Iapichino G, Carenzo L, Cecconi M, Ferrazzi P, Sebastian T, Kucher N, Studt J-D, Sacco C, Alexia B, Sandri MT, Barco S. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. *Thrombosis Research*. 2020; 191: 9-14. https://doi.org/10.1016/j.thromres.2020.04.024.

20  Middeldorp S, Coppens M, van Haars TF, Foppen M, Vlaar AP, Müller MCA, Bouman CCS, Beenens LFM, Kootte RS, Heijmans J, Smits LP, Bonta PI, van Es N. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *Journal of Thrombosis and Haemostasis*. 2020; n/a. 10.1111/jth.14888.
21 Beyrouti R, Adams ME, Cohen H, Farmer SF, Goh YY, Humphries F, Jäger HR, Losseff NA, Perry RJ, Shah S, Simister RJ, Turner D, Chandratheva A, Werring DJ. Characteristics of ischaemic stroke associated with COVID-19. *Journal of Neurology, Neurosurgery &amp; Psychiatry*. 2020: jnnp-2020-323586. 10.1136/jnnp-2020-323586.

22 Andreoli L, Rizzini S, Allegri F, Meroni P, Tincani A. Are the Current Attempts at Standardization of Antiphospholipid Antibodies Still Useful? Emerging Technologies Signal a Shift in Direction. *Semin Thromb Hemost*. 2008; 34: 356-60. 10.1055/s-0028-1085478.

23 Lakos G, Bentow C, Mahler M. A Clinical Approach for Defining the Threshold between Low and Medium Anti-Cardiolipin Antibody Levels for QUANTA Flash Assays. *Antibodies (Basel)*. 2016; 5: 14. 10.3390/antib5020014.

24 Radin M, Cecchi I, Roccatello D, Meroni PL, Sciascia S. Prevalence and Thrombotic Risk Assessment of Anti-β2 Glycoprotein I Domain I Antibodies: A Systematic Review. *Semin Thromb Hemost*. 2018; 44: 466-74. 10.1055/s-0037-1603936.

25 Andreoli L, Chighizola CB, Nalli C, Gerosa M, Borghi MO, Pregnolato F, Grossi C, Zanola A, Allegri F, Norman GL, Mahler M, Meroni PL, Tincani A. Clinical characterization of antiphospholipid syndrome by detection of IgG antibodies against β2-glycoprotein i domain 1 and domain 4/5: ratio of anti-domain 1 to anti-domain 4/5 as a useful new biomarker for antiphospholipid syndrome. *Arthritis & rheumatology (Hoboken, NJ)*. 2015; 67: 2196-204. 10.1002/art.39187.

26 Cattini MG, Bison E, Pontara E, Cheng C, Denas G, Pengo V. Tetra positive thrombotic antiphospholipid syndrome: Major contribution of anti-phosphatidyl-serine/prothrombin antibodies to lupus anticoagulant activity. *Journal of Thrombosis and Haemostasis*. 2020; 18: 1124-32. 10.1111/jth.14765.

27 García-Carrasco M, Galarza-Maldonado C, Mendoza-Pinto C, Escarcega RO, Cervera R. Infections and the Antiphospholipid Syndrome. *Clinical Reviews in Allergy & Immunology*. 2009; 36: 104-8. 10.1007/s12016-008-8103-0.
Cugno M, Meroni PL, Gualtierotti R, Griffini S, Grovetti E, Torri A, Panigada M, Aliberti S, Blasi F, Tedesco F, Peyvandi F. Complement activation in patients with COVID-19: A novel therapeutic target. *J Allergy Clin Immunol.* 2020: S0091-6749(20)30650-3. 10.1016/j.jaci.2020.05.006.

Pregnolato F, Gerosa M, Raimondo MG, Comerio C, Bartoli F, Lonati PA, Borghi MO, Acaia B, Ossola MW, Ferrazzi E, Trespidi L, Meroni PL, Chighizola CB. EUREKA algorithm predicts obstetric risk and response to treatment in women with different subsets of anti-phospholipid antibodies. *Rheumatology.* 2020. 10.1093/rheumatology/keaa203.

Ruben EA, Planer W, Chinnaraj M, Chen Z, Zuo X, Pengo V, De Filippis V, Alluri RK, McCrae KR, Macor P, Tedesco F, Pozzi N. The J-elongated conformation of beta2-glycoprotein I predominates in solution: Implications for our understanding of antiphospholipid syndrome. *J Biol Chem.* 2020. 10.1074/jbc.RA120.013939.
Table 1 – Prevalence of COVID-19 patients positive for aPL

|       | ELISA   | CIA     |
|-------|---------|---------|
|       | aCL     | aβ₂GPI  | aCL CIA | aβ₂GPI CIA |
| IgG   | 5.7 (7)*| 15.6 (19)| 9.8 (12)| 5.0 (6)    |
| IgM   | 6.6 (8) | 9.0 (11)| 6.6 (8) | 5.0 (6)    |
| IgA   | nd      | 6.6 (8) | 2.5 (3) | 0.8 (1)    |

*Values are expressed as percentage (n) of positive patients. aCL: anti-cardiolipin antibodies; aβ₂GPI: anti-β₂ glycoprotein I antibodies; ELISA: enzyme linked immunosorbent assay; CIA: chemiluminescence immunoassay; nd: not done.
Figure 1. Titers of aCL and aβ2GPI antibodies detected by ELISA in COVID-19 patients (black) and comparison with APS patients (green). Values are expressed as median levels [first and third quartile]. Panel A: aCL. From the left to the right: COVID-19 IgG: 15 [8 - 15]; APS IgG: 65 [22 – 103]; COVID-19 IgM: 6.2 [2.6 - 10.8]; and APS IgM: 4.0 [1 - 11]. Panel B: aβ2GPI. From the left to the right: COVID-19 IgG: 0.06 [0.04 – 0.10]; APS IgG: 1.14 [0.52– 1.55]; COVID-19 IgM: 0.065 [0.02 – 0.142]; APS IgM: 0.23 [0.105 – 0.741]; and COVID-19 IgA: 0.04 [0.02 – 0.09]. Cutoff values are aCL IgG/IgM 20 phospholipid units (GPL/MPL); aβ2GPI IgG/IgM/ IgA ELISA 0.13, 0.27 and 0.16 optical units (OD), respectively.
Figure 2

Figure 2. Epitope specificity of anti-β₂GPI antibodies in COVID-19 patients. Panel A. Three-dimensional structure of β₂GPI solved by X-ray crystallography (PDB ID: 6V06 [30]) displaying the positioning of the fragments used in this study. The N-terminal D1 is shown in red. The C-terminal D4-5 fragment is shown in blue. N-linked glycosylations are shown as magenta stick. Titers of anti-D1 (panel B) and anti-D4-5 antibodies (panel C) in 58 COVID-19 patients detected by chemiluminescence and ELISA, respectively. Values are expressed as median levels [first and third quartile]. Anti-D1 (aD1): 3.6 [3.6 – 4.7]. Anti-D4-D5 (aD4-D5): 0.10 [0.068 – 0.199]. Cutoff values are >20 chemiluminescent units (CU) and >0.405 optical units (OD) for aD1 and aD4-D5, respectively.
Figure 3. Titers of aPS/PT antibodies detected by ELISA in COVID-19 patients. Values are expressed as median levels [first and third quartile]. aPS/PT IgG: 13.6 [8 to 15.2]; aPS/PT IgM: 11.4 [8 to 16.5] IgM. Cut-off of the assays was 30 units/ml.