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Beta-2-Glycoprotein-I Deficiency Could Precipitate an Antiphospholipid Syndrome-like Prothrombotic Situation in Patients With Coronavirus Disease 2019

Manuel Serrano,[1] Gerard Espinosa,[2] Antonio Lalueza,[3] Luz Yadira Bravo-Gallego,[2] Raquel Diaz-Simón,[3] Sara Garcinuño,[3] Javier Gil-Etayo,[3] Jorge Moises,[2] Laura Naranjo,[3] Sergio Prieto-González,[2] Estibaliz Ruiz-Ortiz,[2] Beatriz Sánchez,[2] Ana Belen Moreno-Castaño,[2] Carmen Díaz-Pedroche,[3] Odette Viñas-Gomis,[2] Ricard Cervera,[2] and Antonio Serrano,[4] on behalf of the APS-COVID 19 Study Group/European Forum on Antiphospholipid Antibodies

Objective. Patients with coronavirus disease 2019 (COVID-19) present coagulation abnormalities and thromboembolic events that resemble antiphospholipid syndrome (APS). This work has aimed to study the prevalence of APS-related antigens, antibodies, and immune complexes in patients with COVID-19 and their association with clinical events.

Methods. A prospective study was conducted on 474 adults with severe acute respiratory syndrome coronavirus 2 infection hospitalized in two Spanish university hospitals. Patients were evaluated for classic and extra-criteria antiphospholipid antibodies (aPLs), immunoglobulin G (IgG)/immunoglobulin M (IgM) anticardiolipin, IgG/IgM/immunoglobulin A (IgA) anti-β2-glicoprotein-I (aβ2GPI), IgG/IgM antiphosphatidylserine/prothrombin (aPS/PT), the immune complex of IgA aβ2GPI (IgA-aβ2GPI), bounded to β2-glicoprotein-1 (β2GPI) and β2GPI levels soon after COVID-19 diagnosis and were followed-up until medical discharge or death.

Results. Prevalence of aPLs in patients with COVID-19 was as follows: classic aPLs, 5.8%; aPS/PT, 4.6%; IgA-aβ2GPI, 15%; and any aPL, 21%. When patients were compared with individuals of a control group of a similar age, the only significant difference found was the higher prevalence of IgA-aβ2GPI (odds ratio: 2.31; 95% confidence interval: 1.16-4.09). No significant differences were observed in survival, thrombosis, or ventilatory failure in aPL-positive versus aPL-negative patients. β2GPI median levels were much lower in patients with COVID-19 (15.9 mg/l) than in blood donors (168.8 mg/l; $P < 0.001$). Only 3.5% of patients with COVID-19 had normal levels of β2GPI (>85 mg/l). Low levels of β2GPI were significantly associated with ventilatory failure ($P = 0.026$).

Conclusion. β2GPI levels were much lower in patients with COVID-19 than in healthy people. Low β2GPI-levels were associated with ventilatory failure. No differences were observed in the COVID-19 evolution between aPL-positive and aPL-negative patients. Functional β2GPI deficiency could trigger a clinical process similar to that seen in APS but in the absence of aPLs.

INTRODUCTION

Most of the patients with coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1), present an asymptomatic process or mild clinical manifestations. However, slightly less than 15% develop severe manifestations that can be complicated by multiple organ failure and death. Three stages of increasing severity have

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[1]Manuel Serrano, MD, PhD: Hospital 12 de Octubre, Healthcare Research Institute and Hospital Clínico San Carlos, Madrid, Spain; [2]Gerard Espinosa, MD, PhD, Luz Yadira Bravo-Gallego, MD, Jorge Moises, MD, Sergio Prieto-González, MD, PhD, Estibaliz Ruiz-Ortiz, PhD, Beatriz Sánchez, Ana Belen Moreno-Castaño, MD, Odette Viñas-Gomis, MD, PhD, Ricard Cervera, MD, PhD: Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Catalonia, Spain; [3]Antonio Lalueza, MD, PhD, Raquel Diaz-Simón, MD, Sara Garcinuño, BS, Javier Gil-Etayo, BS, Laura Naranjo, BS, Carmen Díaz-Pedroche, MD, PhD: Hospital 12 de Octubre, Healthcare Research Institute I+12, Madrid, Spain; [4]Antonio Serrano, MD, PhD: Hospital 12 de Octubre, Healthcare Research Institute and Biomedical Research Centre Network for Epidemiology and Public Health, Madrid, Spain. See Appendix A for members of the APS-COVID 19 Study Group.

Drs M. Serrano, Espinosa, and Lalueza contributed equally to this work.

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Address correspondence to Antonio Serrano, MD, PhD, Department of Immunology, Hospital 12 Octubre, Av. Cordoba S/N Edificio CAA 5P, 28041 Madrid, Spain. Email: autoimmunity.imas12@h12o.es; aserranoh@gmail.com.

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been identified in COVID-19 (2): 1) nonspecific symptoms, such as fever, malaise, myalgia, and dry cough; 2) pneumonia and acute respiratory distress syndrome with progressive hypoxemia that may require the use of mechanical ventilatory assistance and, histologically, diffuse alveolar damage with intraalveolar fibrin deposition, similar to that seen in influenza virus pneumonia (3); and 3) systemic hyperinflammation in which the process extends to other organs, with elevation of C-reactive protein, ferritin, D-dimer, cytokine, and chemokine levels (4) and a depletion of the immune response with a severe decrease in the T-cell count (effectors and regulators) (5). In March 2020, the mortality rate was at 3.7%, compared to 1% in influenza, 10% in severe acute respiratory syndrome, and 34% in Middle East respiratory syndrome (6).

Patients with COVID-19 with lung or systemic involvement (stages 2 and 3) present coagulation abnormalities, such as prolongation of prothrombin time and activated partial thromboplastin time, increased D-dimer levels, and, in some cases, severe thrombocytopenia (7). These patients are at high risk for thromboembolic events (arterial or venous) and thrombotic microangiopathy (8,9). The incidence of thromboembolic events in patients with COVID-19 is probably underestimated because of the asymptomatic presentation and the failure to perform systematic imaging studies (7). Thrombotic microangiopathy has been found in most of the few autopsies that have been performed to date, and the presence of pulmonary thromboembolism and deep vein thrombosis is striking in many of them (3,10).

This hypercoagulability situation resembles antiphospholipid syndrome (APS), especially in its most severe form, catastrophic APS (11). Zhang et al (12) described a small case series of patients with COVID-19 and thrombotic stroke in which the presence of antiphospholipid antibodies (aPLs) of immunoglobulin A (IgA) and immunoglobulin G (IgG) isotypes was detected; this led to an increase in the interest regarding the role of these antibodies in COVID-19 thrombophilia.

APS classification criteria consider a patient to have thrombotic APS if the thrombosis is accompanied by any of the following aPLs: lupus anticoagulant (LA), IgG or immunoglobulin M (IgM) isotypes of anticardiolipin (aCL), or anti-β2-glycoprotein-I (aβ2GPI) (13). β2-glycoprotein-I (β2GPI), also known as apolipoprotein-H, is one of the major antigenic targets of aPLs. It is an acute phase plasma protein that binds to negatively charged molecules and structures, including anionic phospholipids, heparin, and apoptotic cells (14), and it intervenes in the clearance of apoptotic bodies and viruses from circulation (15,16). Although the exact function of β2GPI has not yet been fully elucidated, it is known that it plays a role in the coagulation cascade, with mainly anticoagulant functions, and it is able to bind to the surface of infectious microorganisms, such as human immunodeficiency virus, rotavirus, and hepatitis B and C viruses (17,18).

In the 16 years since the APS classification criteria have been established, new autoantibodies have been strongly related to APS, but they are still not considered as classification criteria. The main "noncriteria" aPLs are antiphosphatidylserine/prothrombin (aPS/PT) and the aβ2GPI antibodies of the IgA isotype (IgA-aβ2GPI) (19–22).

Although it has been described that up to 87.7% of patients with severe forms of COVID-19 were positive for LA during their stay in the ICU (23), the prevalence and clinical association of the presence of aPLs and other molecules related to APS is not sufficiently known.

The purpose of this work is to study the prevalence of APS-related antigens, antibodies, and immune complexes in patients with COVID-19 and their association with clinical events.

METHODS

Study population and design. A prospective observational study that included 474 hospitalized adult patients diagnosed with COVID-19 in two Spanish tertiary teaching hospitals, one in Madrid (n = 298) and another in Barcelona (n = 176), was conducted. The patients were included consecutively in March 2020 and were followed-up until medical discharge or death.

Control populations. To compare patients with COVID-19 with a healthy population, two control groups were used: 1) healthy anonymous blood donors (n = 228; age range 18-65 years) and 2) a reference group formed by healthy people with an age range similar to that of patients with COVID-19 (n = 131: age range 19-88 years).

Blood donors constitute an excellent reference population; however, this entails a possible bias because people older than 50 years, the most common age range in patients with COVID-19 in our environment, are underrepresented, and those older than 65 years are not included. To minimize this bias, we used the reference group (n = 131) made up of 33 volunteers up to 55 years old who were recruited at the blood donation center and 98 recruited volunteers older than 55 years who underwent a preoperative study for ophthalmic cataract surgery or other minor conditions not related to any major disease. Members of the reference group had no history of serious systemic or vascular pathologies and no symptoms at the time of the medical examination (except for minor age-related symptoms). All members of the control groups were recruited in Madrid before the start of the COVID-19 pandemic.

Study definitions. A COVID-19 case was defined by a positive result for SARS-CoV-2 according to a reverse transcription polymerase chain reaction assay performed on nasal swab sampling from adult patients (older than 18 years) with COVID-19-consistent symptoms who required hospital admission.

Ventilatory failure was defined as an arterial oxygen partial pressure (Pa_{O_2})/fractional inspired oxygen (FI_{O_2}) ratio <200 mm Hg (24) or as the need for mechanical ventilation (either noninvasive positive pressure ventilation or invasive mechanical ventilation).

Poor outcome was defined when at least one of the following criteria was present: 1) ventilatory failure, 2) ICU admission, or 3) death during admission by any cause.

Mortality was defined as patients who died in the first 30 days from the onset of symptoms. This period was considered to guarantee the direct disease causality and avoid the interference of complications arising in prolonged hospitalizations in the ICU.

Hematologic abnormalities included lymphopenia, which was defined as a total lymphocyte count of less than 0.9×10^9/l, and thrombocytopenia, which was defined as a platelet count of less than 150×10^9/l.

Classic aPLs were any of the aPLs included in the updated APS classification criteria (13), excluding LA: aCL or aβ2GPI of IgG/IgM isotypes.

Noncriteria aPLs were aPLs not included in the updated APS classification criteria (13): IgA-aβ2GPI and aPS/PT antibodies of isotypes IgG/IgM.

Laboratory procedures. All the patients were evaluated for aPLs. Most of the serum samples were obtained within the first 24 hours after the presence of the virus was diagnosed.

The classic aPLs (aCL and aβ2GPI of IgG/IgM isotypes) were evaluated by using an antigen coated–beads automatized assay. In Hospital 12 de Octubre, the BioPlex-2200 system (Bio-Rad) was used, and in Hospital Clinic, QUANTA Flash Antiphospholipid Assay Panel by Bioflash (INOVA Diagnostics) was used. The cutoff for BioPlex determinations was 18 U/ml, and the cutoff for Bioflash determinations was 20 U/ml. Both methods are comparable for the evaluation of the classic aPLs (25). The serum samples from the two control groups (blood donors and the reference population) were analyzed at Hospital 12 de Octubre.

The noncriteria aPLs, IgG/IgM aPS/PT and IgA-aβ2GPI, were evaluated in both hospitals by using QUANTA Lite enzyme-linked immunosorbent assay (ELISA) (INOVA Diagnostics). The cutoffs were 30 U/ml, 40 U/ml, and 20 U/ml for aPS/PT IgG, aPS/PT IgM, and IgA-aβ2GPI, respectively, corresponding for each method to the 99th percentile of a healthy population (n = 718). Borderline (grey-zone) results were retested.

The presence of circulating immune complexes (CICs) of IgA bounded to β2GPI was found for the samples of both hospitals with a sandwich ELISA, as previously described. The cutoff was established at 21 UA (26,27).

Serum levels of β2GPI were quantified in a sample of 229 serum samples by using human apoH ELISA^{PRO} kit (Mabtech AB), following the manufacturer's instructions. The mean level described for the general population is in the range of 150 to 300 mg/l (28). Low β2PGI levels were considered at values <85 mg/l, corresponding to previously described mean levels in healthy people (178.3 ± 46.2 g/l) minus twice the SD (29).

ELISA procedures were performed in a Triturus Analyzer (Diagnostics Grifols, S.A.).

Statistical methods. Results of qualitative variables are expressed as absolute frequency and percentage, whereas quantitative variables are expressed as median (interquartile range [IQR]). Association between qualitative variables was determined with Pearson's χ^2 test or Fisher's exact test, when appropriate. The relative measure of an effect is expressed as the odds ratio (OR).

Results of the scaled variables are expressed as median with IQR. The Mann-Whitney *U* test was used for comparisons.

Patients' survival probability and incidence of events were calculated by using the Kaplan-Meier method. The differences between the survival distributions were evaluated with the log-rank test. The relative measure of a condition on survival is expressed as a hazard ratio.

Multivariate analyses were performed by using logistic regression model. Probabilities less than 0.05 were considered significant. Data were analyzed with MedCalc for Windows version 19.3 (MedCalc Software).

Ethical issues. This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of University Hospital 12 de Octubre (reference numbers 20/117, 18/182, and 18/009) and Hospital Clinic (HCB/2020/0727). Oral or written informed consent was obtained from all patients and members of the reference group.

RESULTS

Overall, the median age of the cohort at the moment of the COVID-19 diagnosis was 65 years (IQR: 51-77), and there was a higher proportion of male participants (62.8%). No significant differences in age and sex were observed between patients of both hospitals. No significant differences were observed in the characteristics of the patients (including cardiovascular risk factors) according to the sex (data not shown). Among patients with COVID-19, 112 (23.6%) were positive for at least one aPL, of whom 28 (5.9%) were positive for any of the classic aPLs, 22 (4.6%) were positive for aPS/PT (IgG/IgM), and 71 (14.9%) were positive for IgA-aβ2GPI (Table 1). Only one of them (1.4%) presented immune complexes formed by β2GPI and IgA (immune complexes–positive). The overall characteristics of the cohort and the median levels of each antibody are described in Supplementary Table 1.

The prevalence of aPLs (classic or noncriteria) in patients with COVID-19 was significantly higher than in the group of blood donors (23.6% vs. 6.1%; OR: 4.39; 95% confidence interval [CI]: 2.50-7.73). When we evaluated aPL groups separately, the classic aPL (OR: 3.51; 95% CI: 1.28-10.15) and overall IgA-aβ2GPI (OR: 9.87; 95% CI: 3.56-27.4) groups had a significantly higher prevalence in patients with COVID-19 than in blood donors, but no significant differences were observed in the prevalence of aPS/PT (Table 1). The aPL prevalence in the COVID-19 cohort compared with the control group with a

Table 1. Prevalence of aPLs in patients with COVID-19 (N = 474) and in control populations

	Control	COVID-19	*P*	OR	95% CI
Anonymous blood donors (n = 228)					
Any aPL	15 (6.1%)	112 (23.6%)	<0.001	4.39	2.50-7.73
aCL and/or aβ2GPI IgG/IgM	4 (1.2%)	28 (5.9%)	0.005	3.51	1.28-10.15
aβ2GPI IgA	4 (1.8%)	71 (15.0%)	<0.001	9.87	3.56-27.4
aPS/PT IgG/IgM	7 (3.1%)	22 (4.6%)	0.419	–	–
Reference population (n = 131) of healthy volunteers with an age range similar to that of patients with COVID-19					
Age, median (IQR), years	68 (52-75)	65 (51-77)	0.750	–	–
Sex (female)	66 (50.4%)	202 (42.6%)	0.113	–	–
Any aPL	19 (14.5%)	112 (23.6%)	0.025	1.82	1.07-3.1
aCL and/or aβ2GPI IgG/IgM	4 (3.1%)	28 (5.9%)	0.270	–	–
aβ2GPI IgA	9 (6.9%)	71 (15%)	0.015	2.39	1.16-4.92
aPS/PT	6 (4.6%)	22 (4.6%)	0.977	–	–

Abbreviations: aβ2GPI, anti-β2-glycoprotein-I; aCL, anticardiolipin; aPL, antiphospholipid antibody; aPS/PT, anti-phosphatidylserine/prothrombin; CI, confidence interval; COVID-19, coronavirus disease 2019; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; OR, odds ratio.

similar age range (reference group) was also higher (23.6% vs. 14.5%; OR: 1.82; 95% CI: 1.07-3.1). When we studied aPLs individually, only the prevalence of IgA-aβ2GPI was found significantly higher in patients with COVID-19 (OR: 2.31; 95% CI: 1.16-4.92). No significant differences were found in the prevalence of classic aPLs and aPS/PT between patients with COVID-19 and the reference group (Table 1).

The median level of the β2GPI protein assessed in the serum of patients with COVID-19 was 15.9 mg/l (IQR: 10.8-26.8), this being much lower ($P < 0.001$) than levels observed in blood donors (168.8 mg/l [IQR: 108.1-209.0]) and in the reference group (148.4 mg/l [IQR: 109.3-222.3]) (Figure 1). Only 3.5% of patients with COVID-19 had normal levels of β2GPI (> 85 mg/l). No significant differences were observed in β2GPI levels in patients with COVID-19 by age ($R = 0.108$, $P = 0.102$), sex, or aPL positivity (Supplementary Table 2).

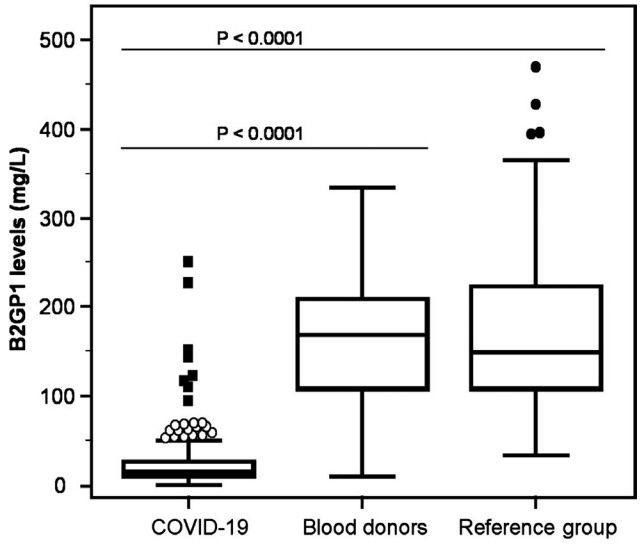

Figure 1. β2-glicoprotein-I (β2GPI) levels in the serum of patients with coronavirus disease 2019 (COVID-19) versus the control groups (blood donors and a reference group). Patients' β2GPI levels were significantly lower than those of both control groups ($P < 0.001$). No significant differences were observed between control groups.

Evolution and outcomes. The median time elapsed between the onset of symptoms and discharge from the hospital was 16 days (IQR: 12-25), and the median time admitted to the hospital was 10.5 days (IQR: 6-18). During their stay in the hospital, 157 (33.1%) patients suffered from ventilatory failure, 35 (7.4%) suffered from thrombotic complications, and 70 (14.8%) died.

The clinical characteristics that were significantly present in patients who suffered ventilatory failure were age (older than 70 years), male sex, and comorbidities, such as diabetes mellitus, dyslipidemia, and arterial hypertension. No significant differences in the prevalence of any aPL and aPL levels were observed (Table 2). Low levels of β2GPI were significantly associated with ventilatory failure. None of the patients with normal β2GPI levels had ventilatory failure, whereas this complication was present in 38.9% (86 of 221) of those with low β2GPI levels ($P = 0.026$). No significant differences were observed in the β2GPI levels of patients who died versus living patients and between patients with thrombosis and patients without thrombosis (Supplementary Table 2).

The patients who died (most of whom had ventilatory failure) presented clinical characteristics similar to those in the patients with ventilatory failure. No differences were found in aPL prevalence between patients who died and survivors (Supplementary Table 3).

No significant differences were observed in the 35 patients who suffered thrombotic events in age, sex, or comorbidities compared with patients without thrombosis (Supplementary Table 4). A significantly higher prevalence of ventilatory failure (OR: 8.02; 95% CI: 3.55-18.12) and classic aPL positivity (OR: 3.01; 95% CI: 1.07-8.49) was found, but in the multivariate analysis, only ventilatory failure behaved significantly as an independent variable (OR: 7.98; 95% CI: 3.52-18.09; coefficient = 2.07). The presence of

Table 2. Comparison of main clinical characteristics according to the ventilatory status

Variable	No ventilatory failure (n = 317)	Ventilatory failure (n = 157)	*P*	OR
Age, years	61 (47.8-75)	71 (61-79)	<0.001	–
Older age (≥70 years)	112 (35.3%)	82 (52.2%)	<0.001	2.0
Sex (female)	151 (47.6%)	51 (32.5%)	0.002	0.53
Dyslipidemia	70 (22.1%)	49 (31.2%)	0.031	1.6
Diabetes mellitus	51 (16.1%)	40 (25.5%)	0.015	1.78
Arterial hypertension	117 (36.9%)	86 (54.8%)	<0.001	2.07
Thrombotic event	8 (2.5%)	27 (17.2%)	<0.001	8.02
Death in 30 days	5 (1.6%)	65 (41.4%)	<0.001	44.05
D-dimer, ng/ml[a]	596 (400-1099)	900 (600-2302)	<0.001	–
Lymphocyte count, 10^9/l[a]	1 (0.8-1.4)	0.7 (0.5-1.0)	<0.001	–
Platelet count, 10^9/l[a]	206 (155-270)	181 (138-250)	0.016	–
C-reactive protein, mg/l[b]	6.5 (2.5-13.7)	15 (8.3-23.5)	<0.001	–
Any aPL	76 (24%)	36 (22.9%)	0.801	–
aCL/aβ2GPI (IgG/IgM)	17 (5.4%)	11 (7%)	0.475	–
aβ2GPI IgA	49 (15.5%)	22 (14%)	0.678	–
β2GPI, mg/l	16 (10.5-31.7)	15.8 (11.6-22.8)	0.598	–
Follow-up, days[c]	14 (11-20)	24 (15-35)	<0.001	–

Note. Variables are expressed as number and percentage or median and interquartile range.
Abbreviations: aβ2GPI, anti-β2-glycoprotein-I; β2GPI, β2-glycoprotein-I; aCL, anticardiolipin; aPL, antiphospholipid antibody; COVID-19, coronavirus disease 2019; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; OR, odds ratio.
[a] Values are stated at onset of COVID-19.
[b] Values are stated at maximum value.
[c] From the onset of symptoms to discharge or death.

classic aPLs lost significance (OR: 2.93; 95% CI: 0.97-8.88; coefficient = 1.07).

Although no case of mortality or thrombosis was observed in patients with normal levels of β2GPI, these results cannot be considered as significant because of the low number of patients with normal values.

Clinical characteristic and outcomes of aPL-positive patients. When assessing the clinical characteristics and outcomes of patients positive for any aPL, no significant differences were observed regarding those without aPLs in thrombosis or ventilatory failure (Table 3), survival (Figure 2A), or time from onset of symptoms to hospital discharge (Figure 2B).

Table 3. Comparison of clinical characteristics, outcomes, and laboratory features according to the status of aPL

Variables	Without aPLs (n = 362)	With aPLs (n = 112)	*P*
Age, years	64 (49-75)	74 (56-83)	<0.001
Older age (≥70 years)	131 (36.2%)	63 (56.3%)	<0.001
Sex (female)	160 (44.2%)	42 (37.5%)	0.210
Dyslipidemia	90 (24.9%)	29 (25.9%)	0.826
Diabetes mellitus	65 (18%)	26 (23.2%)	0.217
Arterial hypertension	147 (40.6%)	56 (50%)	0.079
Ventilatory failure	121 (33.4%)	36 (32.1%)	0.801
Thrombotic event	26 (7.2%)	9 (8%)	0.763
Deceased in 30 days	52 (14.4%)	18 (16.1%)	0.656
Follow-up, days[a]	16 (12-24)	17.5 (12.5-27)	0.733
D-dimers, ng/ml[b]	600 (400-1200)	900 (533-1735)	0.007
Lymphocyte count, 10^9/l[b]	0.9 (0.6-1.3)	1 (0.6-1.2)	0.926
Platelet count, 10^9/l[b]	197.5 (151-62)	190 (150.5-271)	0.978
C-reactive protein, mg/l[c]	8.7 (3.9-15.7)	10.9 (4-17.5)	0.160
Lactate dehydrogenase, U/l[c]	336.5 (275-426)	326 (275.2-413)	0.697
Ferritin, ng/ml[c]	733 (356-1412)	657 (345-1463)	0.921
Troponin, ng/ml[c]	12.5 (6.3-24.9)	16.9 (6.8-36.9)	0.088

Note. Variables are expressed as number and percentage or median and interquartile range.
Abbreviations: aPL, antiphospholipid antibody; COVID-19, coronavirus disease 2019.
[a] From the onset of symptoms to discharge or death.
[b] Values are stated at onset of COVID-19.
[c] Values are stated at maximum value.

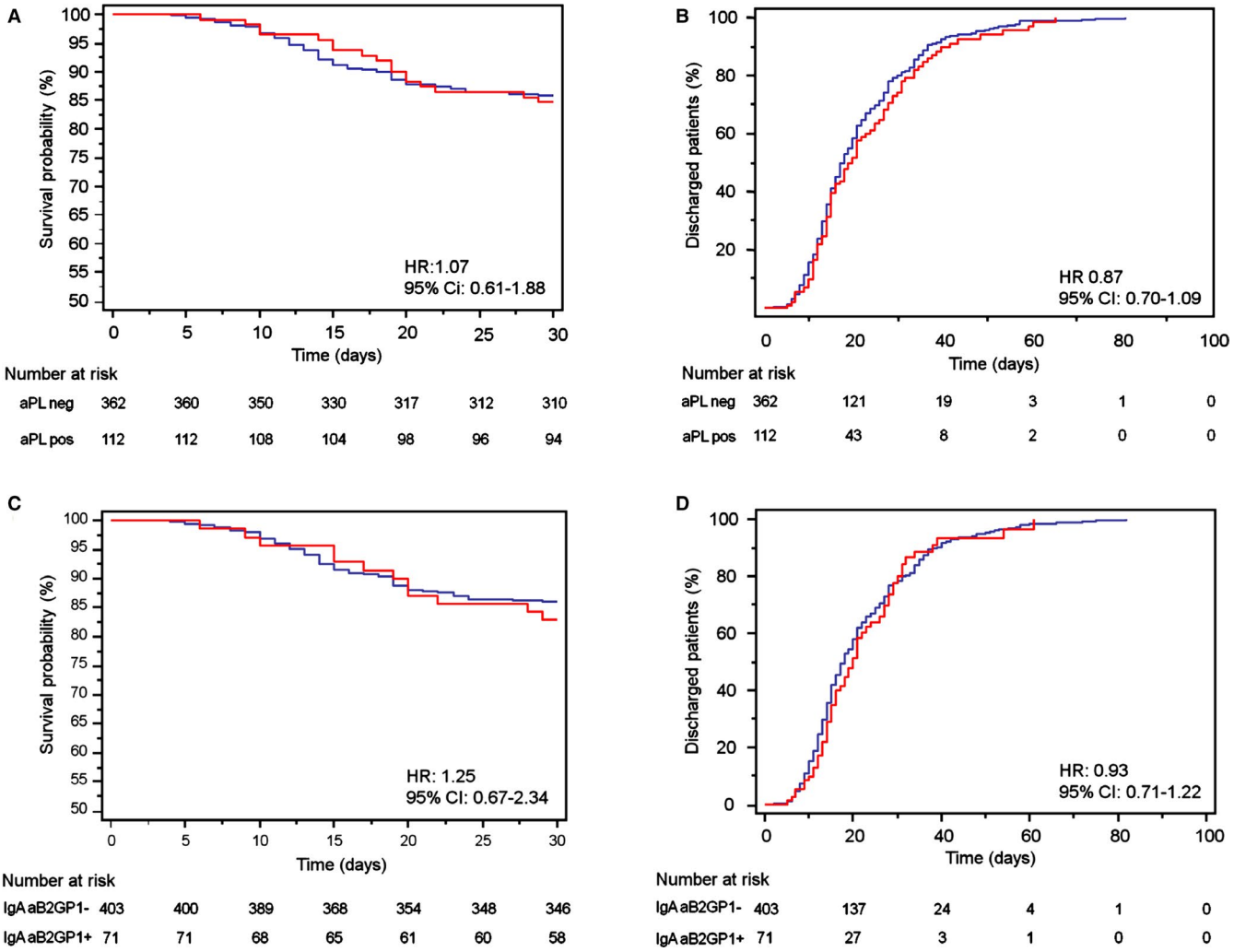

Figure 2. **A**, Comparison of survival between patients with coronavirus disease 2019 (COVID-19) with antiphospholipid antibodies (aPLs) (red) and without aPLs (blue) in accordance with the aPL status. **B**, Comparison of time from symptoms onset to medical discharge in patients with aPLs (red) and without aPLs (blue). **C**, Analysis of survival in patients with COVID-19 who are carriers of the anti-β2-glicoprotein-I antibodies of the immunoglobulin A isotype (IgA-aβ2GPI) (red) and the rest of the patients (blue). **D**, Analysis of time from symptoms onset to hospital discharge in patients with COVID-19 who are IgA-aβ2GPI-positive (red) and the rest of the patients (blue). CI, confidence interval; HR, hazard ratio; neg, negative; pos, positive.

When each subgroup of aPL-positive (aCL/aβ2GPI IgG/IgM, aPS/PT, and IgA-aβ2GPI) were studied separately, no significant differences were found in those positive for aPS/PT or IgA-aβ2GPI (Table 2 and Supplementary Tables 2 and 3). As described above, prevalence of classic aPLs was higher among patients who had thrombosis (5 of 35) than among those without thrombosis (23 of 439; $P = 0.046$).

Because IgA-aβ2GPI was the most prevalent aPL in patients with COVID-19, the characteristics of the carriers of IgA-aβ2GPI versus the rest of the patients were analyzed (Supplementary Table 5). Only significant differences were observed in age (66% of those older than 70 years vs. 36.5%; $P < 0.001$). Survival (Figure 2C) and time from symptom onset to hospital discharge (Figure 2D) did not differ from those observed in the other patients.

The association of aPL levels with the different outputs was also evaluated, and no significant associations were observed (data not shown).

DISCUSSION

We have been able to demonstrate for the first time that blood levels of β2GPI are much lower in patients with COVID-19 than in the general population. Moreover, none of the patients who had normal β2GPI levels had respiratory failure or died. We have also found that the aPL prevalence in patients with COVID-19 is similar to that in controls of the same age (except for IgA-aβ2GPI, whose prevalence is significantly higher) without being associated with the incidence of thrombotic events or other complications of the disease.

In previous studies, we have shown that patients who are IgA-aβ2GPI-positive only developed thrombotic events if they were positive for CICs and that those who were negative for CIC had a similar risk to that of the population without aPLs (27,30). The lack of association of IgA-aβ2GPI with thrombotic events or death would be explained by the practical absence of CIC-positive patients (only 1 of 71 IgA-aβ2GPI-positive patients), which in turn would be a consequence of low antigen levels.

The reason why IgA-aβ2GPI is more prevalent among hospitalized patients with COVID-19 is unknown. Patients with chronic diseases, such as metabolic syndrome or kidney, heart, or liver failure, have a high prevalence of this subtype of aPL (31–33), and these types of chronic diseases are also more prevalent among hospitalized patients with COVID-19 (34). The presence of IgA-aβ2GPI in patients with chronic diseases and elderly people may be related to the elimination of dead cells and apoptotic bodies in a noncomplement-mediated way (minimally inflammatory) because IgA does not fix complement by classical pathway (35).

The mechanisms of response to tissue anoxia and the increase in cell death in the context of SARS-CoV-2 infection suggest the triggering of a prothrombotic situation. The hyaline membrane and the inflammation of the alveolar wall interfere with the correct gas exchange, which may cause local hypoxemia and tissue ischemia. This situation implies, per se, an increased risk of thrombosis (36). In addition, the response to hypoxia involves the formation of hypoxia-inducible transcription factors (HIF-1 and HIF-2) that induce a decrease in protein S levels (antithrombotic), leading to an increase in thrombin levels (37). Likewise, HIF-1 and HIF-2 induce the expression of coagulant factors and integrins that stimulate the formation of prothrombotic extracellular traps of neutrophils and promote the formation of thrombi (38).

Microparticles and cellular debris from apoptotic cells have been reported to facilitate the appearance of thrombosis (39). Cell death involves loss of membrane asymmetry, in which anionic phospholipids, mainly phosphatidylserine, are transferred to the outer membrane mimicking the surface provided by activated platelets (40), facilitating the assembly of the components of prothrombinase. This procoagulant activity is physiologically controlled by β2GPI, which binds to phosphatidylserine, preventing prothrombinase activation (41–43). The apoptotic bodies decorated with β2GPI can be opsonized by aβ2GPI and cleared by macrophages (44), neutralizing their proinflammatory and prothrombotic activity (45).

Blood levels of β2GPI in most of the patients with COVID-19 are more than 10 times lower than that in healthy people. β2GPI gene expression is strongly decreased in patients with COVID-19 (3); therefore, there would be a strong decrease in its production (β2GPI deficiency). Recently, it has been described that patients with sepsis have β2GPI levels 17% lower than controls (mean 165 vs. 198 mg/l) (46). It could be interpreted that the decrease in β2GPI levels in sepsis could be attributed to a higher consumption of the protein, whereas in patients with COVID-19, it could be due to a synergy between higher consumption and lower production.

Lack of β2GPI would impede regulatory function of coagulation and platelet aggregation, leaving patients without weapons to control a thrombotic storm. This situation would be clinically and functionally equivalent to APS, although autoantibodies would not be involved.

It is known that being a carrier of aPLs is not enough to trigger a thrombotic event. A second factor is needed, such as a strong activation of innate immunity (second-hit theory) (47). In aPL carriers, autoantibodies would neutralize only a β2GPI fraction (open form), leaving enough protein available to fulfil its physiological functions. Faced with an overload situation (second hit) with a higher consumption of β2GPI (surgery or infection), the protein levels would be insufficient to block thrombus formation. Lack of sufficient functional β2GPI would be a common pathogenetic mechanism of thrombus formation in COVID-19 and APS.

However, partial β2GPI deficiency (presence of low levels of protein) has been related to thrombosis. Recently, Zhang el al (48) described that a mutation in the *APOH* gene impairing β2GPI production was associated with recurrent thrombosis. This work confirms previous observations that described that patients with the β2GPI H3 haplotype (the one with lower plasma protein levels) have a higher thrombin generation capacity, along with a seven times greater risk of venous thrombosis, than β2GPI H1 haplotype carriers (the most common haplotype present in 85%-90% of the population) (49).

COVID-19 would be similar in behavior to an acquired β2GPI deficiency that would be triggered at the time of infection. On the functional level, the deficiency of β2GPI would be equivalent to the antibody-mediated blockade that occurs in APS: an APS-like syndrome that is really seronegative. Likewise, in the case of patients with COVID-19 with prothrombotic aPLs (50), this effect would be amplified by the β2GPI deficiency. A deeper study of hypercoagulability related to β2GPI deficiency, congenital or in the context of infections, could help to better understand the pathogenic mechanisms underlying APS, seen from another perspective.

This work has several limitations. Firstly, the LA was not incorporated into the analysis. It would be interesting to compare LA activity in patients with very low β2GPI levels. The highly overloaded hospital situation during the pandemic and the widespread use of COVID-19 anticoagulant treatment, which could interfere with the interpretation of LA test results, meant that many tests, such as LA, could only be conducted in a minority of the patients. Secondly, only an initial sample was available, and therefore we lack information about the evolution of β2GPI values or the persistence of aPLs over time. In subsequent studies, the evolution of β2GPI levels over several weeks and their relationship with the clinical improvement of patients or late thrombosis should be addressed. The use of different assays to evaluate classic aPLs is another limitation; however, the results with both diagnostic systems are not significant. For the noncriteria aPLs, which are those

that show significant differences in patients with COVID-19 versus the general population, the same methodology was used in both centers. Another limitation is that aPL levels can be dynamic and aPLs could emerge later in some patients. Conducting new studies to evaluate possible variations in the titer of aPLs during the first months from the onset of infection is mandatory. Finally, the fact that only hospitalized patients were included is another limitation because patients with less severe forms of COVID-19 were underrepresented and we have not been able to confirm if the patients with normal levels of β2GPI would present a better evolution. The study of hospitalized patients, per se, is an element of confounding because these patients have a higher incidence of thrombotic events because of immobility.

If this finding is corroborated, prophylactic treatment of patients with fresh plasma (an indirect way to replenish β2GPI) or parenteral β2GPI could be considered to avoid COVID-19 complications. In this way, therapeutic plasma exchange (TPE) by using normal plasma (not from patients who have recovered from COVID-19) allows for a rapid improvement of patients with COVID-19 in general condition and of Pa_{O_2}/FI_{O_2} that is clearly seen within 24 hours (51–53). This positive effect of TPE has also been described in patients who are aPL carriers (54).

New multicenter studies that also include mild cases are needed to confirm the value of the absence of β2GPI in the context of COVID-19, especially as a function of time, and clinical trials are needed to determine the possible therapeutic value of B2GPI replacement therapies in COVID-19 and other extreme stressors, such as sepsis or acute respiratory distress syndrome.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. A. Serrano had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. M. Serrano, Espinosa, Lalueza, Prieto-González, Cervera, A. Serrano.

Acquisition of data. Espinosa, Lalueza, Diaz-Simón, Prieto-González, Sánchez, Díaz-Pedroche, members of APS-COVID 19 Study Group/European Forum on Antiphospholipid Antibodies.

Analysis and interpretation of data. M. Serrano, Espinosa, Lalueza, Prieto-González, Viñas-Gomis, Cervera, A. Serrano.

Laboratory work. Bravo-Gallego, Garcinuño, Gil-Etayo, Moreno-Castaño, Naranjo, M. Serrano, Viñas-Gomis.

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APPENDIX A: APS-COVID 19 STUDY GROUP/EUROPEAN FORUM ON ANTIPHOSPHOLIPID ANTIBODIES MEMBERS

Members of the APS-COVID 19 Study Group/European Forum on Antiphospholipid Antibodies. Members are as follows (sorted alphabetically by team and last name): team 1 (Barcelona): Alex Almuedo,[1] Luz Bravo-Gallego,[2] Daniel Camprubí,[1] Júlia Calvo,[3] Aina Capdevila-Reniu,[3] Irene Carbonell,[3] Ricard Cervera,[4] Georgina Espígol-Frigolé,[4] Gerard Espinosa,[4] Irene Fuertes,[5] Cristina Gabara,[3] Priscila Giavedoni,[5] Ignacio Grafia,[3] Andrea Ladino,[3] Gema María Lledó-Ibáñez,[4] Ana Matas-García,[3] Pere Millat,[1] Pedro Juan Moreno,[3] Jorge Moises,[6] Ana Belen Moreno-Castaño,[7] Magdalena Muelas,[1] José Muñoz,[1] José Naval,[3] Joan Padrosa,[3] Martina Pellicé,[3] María Jesús Pinazo,[1] Sergio Prieto-González,[4] Roberto Ríos-Garcés,[4] Natalia Rodríguez,[1] Olga Rodríguez-Núñez,[2] Estibaliz Ruiz-Ortiz,[2] Ruth Sotil,[1] Adrià Tomé,[4] Helena Ventosa,[3] Odette Viñas-Gomis,[2] and Carles Zamora-Martínez,[3]; team 2 (Madrid): Luis Allende,[8] Estíbaliz Arrieta,[9] Oscar Cabrera-Marante,[8] Cristina de la Calle,[9] María José Castro,[8] Carmen Díaz-Pedroche,[9] Raquel Diaz-Simón,[9] Dolores Folgueira,[10,11] Ana García-Reyne,[9] Sara Garcinuño,[8] Javier Gil-Etayo,[8] Rocío Laguna,[8] Antonio Lalueza,[9] Elena Ana López,[12] Jaime Lora-Tamayo,[9,11] Carlos Lumbreras,[9,11] Guillermo Maestro-de la Calle,[9] Esther Mancebo,[8] Mikel Mancheño-Losa,[9] Álvaro Marchán-López,[9] Borja de Miguel-Campo,[9] Pablo Morales,[8] Laura Naranjo,[8] Estela Paz-Artal,[8] Daniel Pleguezuelo,[8] Edgard Rodríguez,[8] Antonio Serrano,[8] Manuel Serrano,[13] and Paloma Talayero.[8]

1. Department of Tropical Medicine and International Health, ISGlobal, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain.
2. Department of Immunology, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.
3. Department of Internal Medicine, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.
4. Department of Autoimmune Diseases, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.
5. Department of Dermatology, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.
6. Department of Pneumology, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Catalonia, Spain.
7. Department of Pathology, Center for Biomedical Diagnosis. Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Catalonia, Spain.
8. Department of Immunology, University Hospital 12 de Octubre, Healthcare Research Institute, Madrid, Spain.
9. Department of Internal Medicine, University Hospital 12 de Octubre, Healthcare Research Institute, Madrid, Spain.
10. Department of Medicine, School of Medicine, Complutense University, Madrid, Spain.
11. Department of Microbiology, University Hospital 12 de Octubre and Healthcare Research Institute, Madrid, Spain.
12. Department of Biochemistry, University Hospital 12 de Octubre, Madrid, Spain.
13. Department of Immunology, University Hospital Clinico de San Carlos, Madrid, Spain.