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Immunogenicity, safety, and antiphospholipid antibodies after SARS-CoV-2 vaccine in patients with primary antiphospholipid syndrome

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Abstract

Objective: Coronavirus disease 19 (COVID-19) has an increased risk of coagulopathy with high frequency of antiphospholipid antibodies (aPL). Recent reports of thrombosis associated with adenovirus-based vaccines raised concern that SARS-CoV-2 immunization in primary antiphospholipid syndrome (PAPS) patients may trigger clotting complications. Our objectives were to assess immunogenicity, safety, and aPL production in PAPS patients, after vaccinating with Sinovac-CoronaVac, an inactivated virus vaccine against COVID-19.

Methods: This prospective controlled phase-4 study of PAPS patients and a control group (CG) consisted of a two-dose Sinovac-CoronaVac (D0/D28) and blood collection before vaccination (D0), at D28 and 6 weeks after second dose (D69) for immunogenicity/aPL levels. Outcomes were seroconversion (SC) rates of anti-SARS-CoV-2 S1/S2 IgG and/or neutralizing antibodies (NAb) at D28/D69 in naïve participants. Safety and aPL production were also assessed.

Results: We included 44 PAPS patients (31 naïve) and 132 CG (108 naïve) with comparable age (p=0.982) and sex (p>0.999). At D69, both groups had high and comparable SC (83.9% vs. 93.5%, p=0.092), as well as NAb positivity (77.4% vs. 78.7%, p=0.440), and NAb-activity (64.3% vs. 60.9%, p=0.689). Thrombotic events up to 6 months or other moderate/severe side effects were not observed. PAPS patients remained with stable aPL levels throughout the study at D0 vs. D28 vs. D69: anticardiolipin (aCL) IgG (p=0.058) and IgM (p=0.091); anti-beta-2 glycoprotein I (aβ2GPI) IgG (p=0.513) and IgM (p=0.468).

Conclusion: We provided novel evidence that Sinovac-CoronaVac has high immunogenicity and safety profile in PAPS. Furthermore, Sinovac-CoronaVac did not trigger thrombosis nor induced changes in aPL production.

Keywords

COVID-19, vaccine immunogenicity, SARS-CoV-2 vaccine, antiphospholipid syndrome, antiphospholipid antibodies

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Background

Coronavirus disease 19 (COVID-19) has an increased risk of coagulopathy, especially the occurrence of thromboembolic events. The intense inflammatory response evoked by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication may induce a dysregulation of coagulation toward a hypercoagulable state,[1-3] and both large vessels and microcirculation may be affected.[4-6]

Antiphospholipid syndrome (APS) is the most frequent acquired thrombophilia.[7] Half of the cases, known as primary APS (PAPS), occur without the concomitance of other autoimmune rheumatic diseases (ARD).[8] APS is characterized by the persistent presence of antiphospholipid antibodies (aPL), namely, lupus anticoagulant (LA), IgG, and/or IgM aCL and IgG and/or IgM aβ2GPI, which play an important role in the pathogenesis of thrombosis in those patients.[9]

Interestingly, both diseases share common mechanisms of thrombosis: activation of endothelial cells, resulting in inhibition of endothelial nitric oxide synthase production, and consequently, decreasing nitric oxide production; complement activation; and unchecked inflammatory signals responsible for the formation of neutrophil extracellular traps (NETosis).[10]

Recent studies reported the presence of aPL in patients infected with SARS-CoV-2.[11-14] Furthermore, infectious etiologies may act as the "second hit," crucial for the thrombogenesis in APS and/or aPL-positive patients.[15-16] Therefore, vaccinating these patients to prevent COVID-19 is of utmost importance.

Paradoxically, two of the vaccines against SARS-CoV-2 using adenovirus platforms developed by AstraZeneca and Janssen have been associated with the occurrence of rare and atypical thromboembolic events, especially in women under 50 years of age, a condition that has been called vaccine-induced immune thrombotic thrombocytopenia (VITT).[17,18] As a consequence, vaccinating patients with thrombophilia using other platforms, such as inactivated virus or mRNA, may be preferable in this subset of patients. However, studies on the efficacy and safety of those vaccines in APS are still lacking.

CoronaVac (Sinovac Life Sciences, Beijing, China) is an inactivated vaccine against COVID-19, which is supporting vaccination campaigns in more than 40 countries, including Brazil, and has shown good tolerance and efficacy in inducing humoral responses against SARS-CoV-2 in the general population.[19-21] Jara et al.[22] demonstrated that Sinovac-CoronaVac reduced rates of infection, hospitalization, ICU admission, and death by 65.9%, 87.5%, 90.3%, and 86.3%, respectively, in the overall population of 10.2 million people in Chile.

The aims of the present prospective study were to evaluate immunogenicity of Sinovac-CoronaVac vaccine in naïve PAPS patients compared to a balanced age- and sex-control group (CG). We further assessed safety, including thrombotic events, and the possible vaccine-induced aPL production throughout the study period.

Methods

Study design

This study is a subgroup analysis of patients with PAPS from a large phase four prospective controlled trial with ARD patients performed at a single tertiary center in Brazil.[23]

Patients and controls

All consecutive PAPS patients who fulfilled the current classification criteria for PAPS (Sidney) [9] and were regularly followed in our Outpatient Rheumatology Clinics and were ≥18 years old were invited to participate. Subsequently, a CG of hospital maintenance, administrative personal, or their relatives balanced by sex and age (±5 years differences) using an Excel program (ratio 1PAPS: 3CG) were also invited to participate. Exclusion criteria for both groups were the following: ARD (other than APS, for the patient's group), use of immunosuppressive drugs, HIV infection, history of anaphylactic response to vaccine components, acute febrile illness or symptoms compatible to COVID-19 at vaccination, previous demyelinating disease (including Guillain-Barré syndrome), symptomatic heart failure (class III or IV), previous vaccination with any SARS-CoV-2 vaccine, history of vaccination with live virus vaccine in the previous 4 weeks or with virus vaccine inactivated in the previous 2 weeks, history of having received blood products in the previous 6 months, individuals who refused to participate in the study, and hospitalized patients.

Participants who developed RT-PCR-confirmed COVID-19 after receiving the first vaccine dose (incident cases) and with positive COVID-19 serology and/or NAb at baseline (collected on the day of vaccination) were excluded from the immunogenicity and aPL analysis; however, they were included in the safety evaluation.

Vaccine protocol

PAPS patients and CG were scheduled to receive a two-dose vaccine. The first dose was given on February 9–18th 2021 (D0, with baseline blood collection immediately before it); the second dose was given 28 days later (D28, with blood collection immediately before it). A third blood sample was obtained 6 weeks after the second dose at day 69 (D69). This protocol was delayed 4 weeks for participants with incident COVID-19 infection during the study. Ready-to-use

syringes loaded with CoronaVac (Sinovac Life Sciences, Beijing, China, batch #20200412), that consists of 3 µg in 0.5 mL of β-propiolactone inactivated SARS-CoV-2 (derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells—Vero 25 cells) with aluminum hydroxide as an adjuvant, were administered intramuscularly in the deltoid area. The sera of each blood sample (20 mL) from all participants obtained at days D0, D28, and D69 were stored in a −70°C freezer.

Anti-SARS-CoV-2 S1/S2 IgG antibodies

A chemiluminescent immunoassay was used to measure human IgG antibodies against the S1 and S2 proteins in the receptor binding domain (RBD) (Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). Seroconversion rate (SC) was defined as positive serology (>15.0 UA/mL) post vaccination, since only patients with pre-vaccination negative serology were included. Geometric mean titers (GMT) of these antibodies and 95% confidence intervals were also calculated at all time points, attributing the value of 1.9 UA/mL (half of the lower limit of quantification 3.8 UA/mL) to undetectable levels (<3.8 UA/mL). The factor increase in GMT (FI-GMT) is the ratio of the GMT after vaccination to the GMT before vaccination, used to demonstrate growth in IgG titers. They are also presented and compared as geometric means and 95% confidence intervals (CI).

SARS-CoV-2 cPass virus-neutralization antibodies

The SARS-CoV-2 sVNT Kit (GenScript, Piscataway, NJ, USA) was performed according to manufacturer instructions. This analysis detects circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the RBD of the viral spike glycoprotein with the ACE2 cell surface receptor. The tests were performed on the ETI-MAX-3000 equipment (DiaSorin, Italy). The samples were classified as either "positive" (inhibition ≥30%) or "negative" (inhibition <30%), as suggested by the manufacturer.[24] The frequency of positive samples was calculated at all time points. Medians (interquartile range) of the percentage of neutralizing activity only for positive samples were calculated.

Outcomes

Immunogenicity outcome was assessed by two criteria SC rates of total anti-SARS-Cov-2 S1/S2 IgG and presence of NAb at D69. Other endpoints were the following: anti-S1/S2 IgG SC and presence of NAb at D28 (after vaccine first dose); geometric mean titers of anti-S1/S2 IgG and their FI-GMT at D28 and D69; and median (interquartile range) neutralizing activity of NAb at D28 and D69.

Vaccine adverse events and incident cases of COVID-19

Patients and CG were advised to report any side effects of the vaccine. They received on D0 (first dose) and on D28 (second dose) a standardized diary for local and systemic manifestations. The standardized diary of adverse events (AE) was carefully reviewed with each participant on the day of the second dose (D28) and at the last visit (D69). COVID-19 incident cases were followed for 40 days (from D0 to 10 days after the second dose [D39]) and thereafter for the following 40 days (from D40 to D79).

Vaccine AE severity was defined according to WHO definitions.[25] A rigorous surveillance for any kind of thrombotic event was performed during a period of 6 months after full-vaccination.

Additionally, all participants were instructed to communicate any manifestation associated or not with COVID-19 through telephone, smartphone instant messaging, or email. Suspicious cases of COVID-19 were instructed to seek medical care near the residence and, if recommended, to come to our tertiary hospital to have the RT-PCR exam or in-person visit. Patients were clinically followed for 6 months (August 18, 2021).

Study data were collected and managed using REDCap electronic data capture tools hosted at our Institution.[26-27]

RT-PCR for SARS-CoV-2

Clinical samples for SARS-CoV-2 RT-PCR consisted of nasopharyngeal and oropharyngeal swabs, using a laboratory developed test.[28]

Antiphospholipid antibodies

We assessed the criteria antiphospholipid antibodies IgG/IgM aCL and IgG/IgM anti-β2GPI in PAPS patients. Peripheral blood samples were collected in dry tubes (2 tubes), respecting the time between collection and centrifugation of at most 1 hour. Samples were centrifuged at 3200 r/min for 15 min and aliquoted in a volume of 500 µL. The aCL antibodies were detected by commercial fluoro immunoenzymatic assay (EliA) Thermo Scientific™/Phadia™ 250 Immunoassay Analyzers and they were considered positive if present in medium or high titers (≥40 GPL or MPL). The aβ2GPI antibodies were measured through the enzyme-linked immunosorbent assay (ELISA) QUANTALite®, InovaDiagnostics and their positivity was defined if titers were > 20UI/mL. Antiphospholipid antibodies at D28 and D69 were compared to baseline (D0) to verify if there was any increase in titers after vaccination. The thrombosis score risk aGAPSS (adjusted Global AntiPhospholipid Syndrome Score) that includes the three criteria aPL,[29-30] besides arterial hypertension and

dyslipidemia, was calculated at baseline and at D69 using LA previously registered in our electronic database. LA detection was performed according to updated guidelines.[31]

Statistical analysis

A convenience sample of PAPS patients was selected with a CG in a 1:3 ratio. Continuous variables are presented as medians (interquartile ranges) with intergroup comparison using Mann–Whitney test. Categorical variables are presented as number (percentage) and compared using chi-square or Fisher's exact tests, as appropriate. Continuous data regarding anti-S1/S2 serology titers are presented as geometric means (95% CI) and compared with the same tests, but in Napierian logarithm (ln) transformed data. Longitudinal comparisons of ln-transformed anti-S1/S2 IgG titers between PAPS and CG were performed using generalized estimating equations (GEE) with normal marginal distribution and gamma distribution, respectively. Results were followed by Bonferroni multiple comparisons to identify differences between groups and time points. Multivariate logistic regression analyses were performed using as dependent variables SC or presence of NAb, and as independent variables those with p <0.2 in univariate analysis. The isotypes of each aPL were analyzed categorically (according to aPL cutoff positivity definitions) using Chi-square test and continuously by Friedman Repeated Measures Analysis of Variance on Ranks at D0, D28, and D69. aGAPSS score of APS patients was also compared between the three time points using Friedman Repeated Measures Analysis of Variance on Ranks.

Statistical significance was defined as p <0.05. All statistical analyses were performed using IBM-SPSS for Windows software version 22.0.

Ethics statement

The protocol was approved by the National and Institutional Ethical Committee of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), Brazil (CAAE: 42566621.0.0000.0068). It was in accordance with the Declaration of Helsinki and local regulations, and all participants signed a written informed consent before enrollment.

Results

Participants

We initially selected 63 patients, but six patients did not attend the vaccine appointment, one patient had symptoms compatible with COVID-19 at the day of vaccination and 12 patients had associated systemic lupus erythematosus (SLE) and were excluded. The remaining 44 PAPS patients and 132 controls were included in the study. Forty-three patients had thrombotic criteria (97.7%) and 18 (40.9%) had obstetric criteria. Only one patient was classified as exclusively obstetric. Triple positivity was present in 45.4% of cases (Table 1). The number of triple positives was even higher (54.8%) considering only the 31 naïve-PAPS.

PAPS patients and CG had comparable median ages (46 [31–73] vs. 46 [31–78] years, p=0.982) and female sex (86.4% in both groups, p=1.0) at study entry. The mean duration of disease in PAPS patients was 16.7 ± 8.4 years. Of note, the PAPS group had more stroke than CG (29.5% vs. 0%, p<0.001), besides dyslipidemia (59.1% vs. 8.3%, p<0.001) and smoking (38.6% vs. 8.3%, p<0.001). These characteristics are shown in Table 1.

Vaccine immunogenicity

For this analysis, we excluded 37 (21.0%) participants (13 PAPS patients and 24 CG) due to pre-vaccination positive COVID-19 serology (6 PAPS and 18 CG) and/or NAb (1 PAPS and 3 CG) and the incidents confirmed cases of COVID-19 during the study (1 PAPS and 3 CG). Further exclusions for the immunogenicity analyses were related to continuous immunosuppression (not related to APS): two patients were using azathioprine and prednisone (one due to autoimmune hepatitis and the other due to idiopathic interstitial pulmonary disease); one patient with renal transplant was on mycophenolate mofetil, tacrolimus, and prednisone; one patient with cardiac transplant was on mycophenolate mofetil and cyclosporine; and one patient was using prednisone to treat livedoid vasculopathy.

The final immunogenicity analysis included 31 naïve-PAPS patients and 108 controls. Flow chart of the study is illustrated in Figure 1.

Anti-SARS-CoV-2 IgG antibodies

There was a modest initial response of anti-SARS-CoV-2 IgG in both groups after the first dose with comparable SC in naïve-PAPS patients a CG at D28 (25.8% vs. 30.6%, p=0.609). The SC rates at D69 increased approximately 3-fold after the second dose with similar immunogenicity for naïve-PAPS and GC groups: SC rates (83.9% vs. 93.5%, p=0.092) and geometric mean titers (GMT) (50.2 [95%CI 34.5–73.2] in PAPS vs. 61.7 [95%CI 52.8–72.3] in CG, p=0.249). The factor increase in GMT (FI-GMT) at D69 was also elevated in naïve-PAPS and CG (21.4 [95%CI 14.5–31.6] vs. 26.5 [95%CI 22.3–31.4], p=0.586) and at D69, respectively (Table 2).

According to Bonferroni's multiple comparison, there was a significant GMT increase when we performed longitudinal comparisons of GMT in naïve-PAPS patients at baseline versus D28 and D69 (p<0.001, for both) and at D28 vs. D69 (p<0.001). Likewise, the results of longitudinal

GMT comparisons in CG at D28 and D69 vs. baseline and between D69 vs. D28 also showed a significant increase (p<0.001, for all comparisons) (Table 2).

SARS-CoV-2 cPass virus-neutralization antibodies (NAb)

The frequency of NAb at D28 was lower in naïve-PAPS patients than CG (16.1% vs. 35.2%, p=0.043), with a robust rise at D69 and comparable NAb positivity rates among both groups (77.4% vs. 78.7%, p=0.440). NAb-activity was comparable in naïve-PAPS patients and CG at D28 (38.1% [32.0–55.5] vs. 43.7% [34.2–66.4], p=0.275) and D69 (64.3 [49.0–77.0%] vs. 60.9 [45.6–81.3%], p=0.689) (Table 3).

Antiphospholipid antibodies and vaccination

High titers of aCL at baseline were identified in 13/31 (41.9%) of the naïve-APS patients (seven of IgG isotype, four of IgM isotype, and 1 with both isotypes). Fourteen (45.2%) patients had high titers of aβ2GPI at baseline (four with IgG isotype, eight of IgM isotype, and two with both isotypes). All patients remained positive for aCL and/or aβ2GPI without significant changes in titers, but one patient with negative IgM aCL (5 MPL) and IgM aβ2GPI (5 UI/mL) at baseline and at D28 (IgM aCL: four MPL and IgM aβ2GPI:4 UI/mL) had an increment to 48 MPL and 42 UI/mL, respectively, at day 69.

No significant difference was found between samples collected before and after vaccination for all four autoantibodies (Figure 2). In the quantitative analysis, titers remained stable over time. In the qualitative assessment, frequencies of positivity also did not change for all aPL: IgG aCL positivity rates were 25.8% (n=8/31) vs. 25.8% (n=8/31) vs. 22.6% (n=7/31), p=0.944, at D0, D28, and D69; IgM aCL positivity rates were 16.1% (n=5/31) vs. 16.1% (n=5/31) vs. 19.4% (n=6/31), p=0.927, at D0, D28, and D69; IgG aβ2GPI positivity rates were 12.9% (n=4/31) vs. 12.9% (n=4/31) vs. 16.1% (n=5/31), p=0.914, at D0, D28, and D69; and IgM aβ2GPI positivity rates were 16.1% (n=5/31) vs. 16.1% (n=5/31) vs. 19.4% (n=6/31), p=0.927, at D0, D28, and D69.

The median (interquartile range) aGAPSS of the 31 naïve-APS patients did not modify after completing vaccination (D0 vs D28 vs D69: 13 [4–17] vs. 13 [4–17] vs. 13 [4–17], p=0.717).

Vaccine safety and tolerance

We did not observe any moderate/severe AE in any group. Local and systemic reactions were more common in the PAPS group after the first dose compared to controls, but not after the second dose. The overall description of AE in PAPS patients and controls is summarized in Table 4.

Table 1. Baseline characteristics of primary antiphospholipid syndrome patients and controls.

	PAPS (n=44)	Controls (n=132)	p-Value
Demographics			
Current age, years	46 (31–73)	46 (31–78)	0.982
Age at diagnosis, years	29 (17–67)	-	-
Disease duration, years	16.7 ± 8.4	-	-
Female sex	44 (86.4)	114 (86.4)	>0.999
Caucasian race	27 (61.4)	64 (48.5)	0.139
Comorbidities			
Systemic arterial hypertension	18 (40.9)	39 (29.5)	0.163
Diabetes mellitus	3 (6.8)	16 (12.1)	0.411
Dyslipidemia	26 (59.1)	11 (8.3)	<0.001
Obesity	21 (47.7)	42 (32.3)	0.066
Current smoking	17 (38.6)	11 (8.3)	<0.001
APS criteria manifestations			
Thrombotic	43 (97.7)	-	-
Arterial	21 (47.7)	-	-
Stroke	13 (29.5)	0 (0)	<0.001
Venous	25 (56.8)	-	-
Obstetric	18 (40.9)	-	-
aPL profile			
Single positivity	11 (25.0)	-	-
Double positivity	13 (29.5)	-	-
Triple positivity	20 (45.5)		
APS treatment			
VKA	39 (88.6)	-	-
LMWH	3 (6.8)	-	-
LDA	8 (18.2)	-	
Hydroxychloroquine	17 (38.6)	-	

Results are expressed in mean ± standard deviation, median (minimum and maximum values), and n (%).
PAPS—primary antiphospholipid syndrome; aPL—antiphospholipid antibody; VKA—vitamin K antagonist; LMWH—low-molecular-weight heparin; LDA—low dose aspirin.

COVID-19 incident cases

During the study, four participants (one PAPS patient and three CG) had incident symptomatic cases of COVID-19, all confirmed by RT-PCR. All cases occurred from D0 to D32 and none of them was hospitalized.

Discussion

To the best of our knowledge, this is the first study to demonstrate that the Sinovac-CoronaVac vaccine is highly immunogenic and safe in PAPS patients and did not trigger short- and medium-term thrombosis or increase of aPL-related antibodies production.

Recent studies focusing on an overall evaluation of mRNA COVID-19 immunized ARD patients have shown a

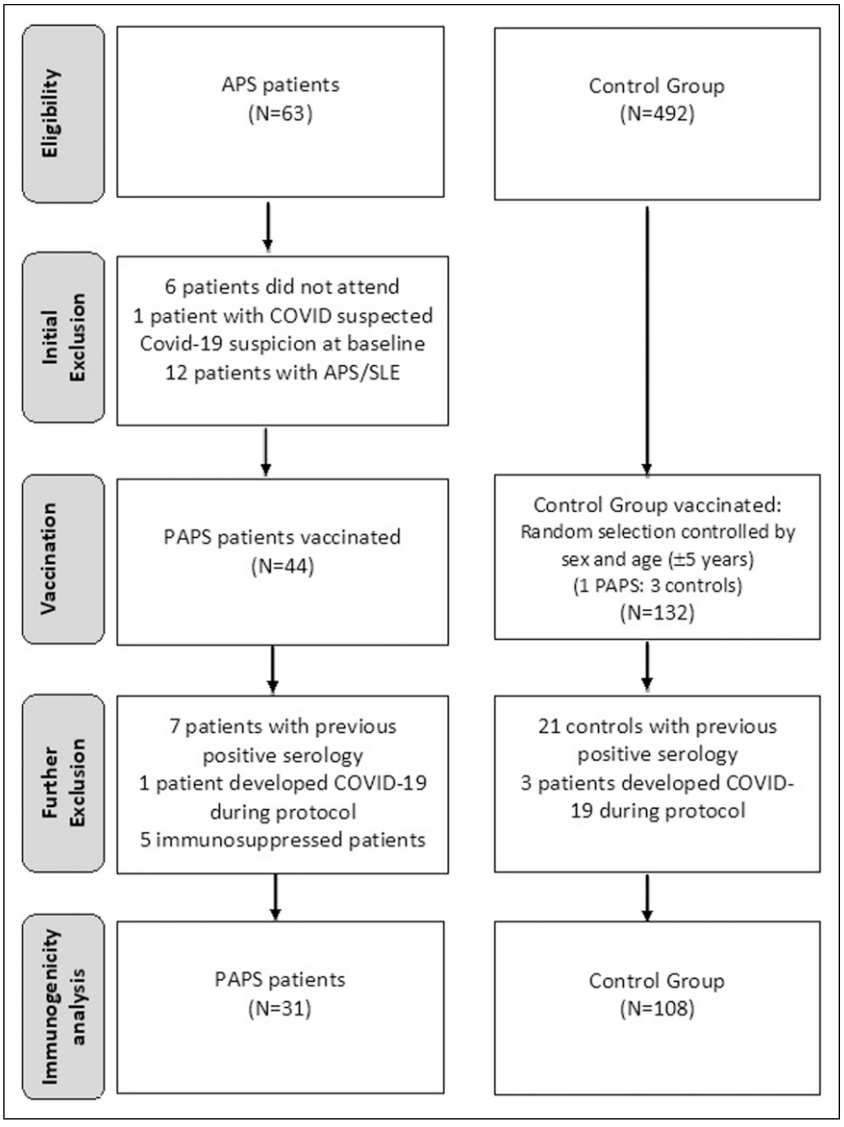

Figure 1. Flowchart of patients and controls submitted to Sinovac-CoronaVac vaccination.

Table 2. Seroconversion rates and anti-SARS-CoV-2 S1/S2 IgG titers before and after CoronaVac in näive-PAPS and controls.

	Seroconversion (SC)		Geometric mean titer (GMT)			Factor increase in GMT	
	D28	D69	D0	D28	D69	D28	D69
PAPS, *n*=31	8 (25.8)	26 (83.9)	2.4 (2.0–2.7)	7.7 (5.1–11.6)[a]	50.2 (34.5–73.2)[a,b]	3.3 (2.2–4.9)	21.4 (14.5–31.6)
Controls, *n*=108	33 (30.6)	101 (93.5)	2.3 (2.1–2.6)	9.8 (7.6–12.6)[c]	61.7 (52.8–72.3)[c,d]	4.2 (3.4–5.1)	26.5 (22.3–31.4)
p-Value (PAPS vs CG)	0.609	0.092	0.936	0.359	0.249	0.600	0.586

PAPS—Primary antiphospholipid syndrome; CG—control group; SC—Seroconversion (defined as post-vaccination titer >15 AU/mL—Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy); GMT—Geometric mean titers (AU/mL).
Frequencies of SC are presented as number (%) and they were compared using chi-square between PAPS patients and CG at pre-specified time points (D28 and D69). IgG antibody titers and FI-GMT are expressed as geometric means with 95% confidence interval (95%CI). Comparisons of ln-transformed anti-S1/S2 IgG titers between PAPS and CG were performed using generalized estimating equations (GEE) with normal marginal distribution and gamma distribution, respectively. Results were followed by Bonferroni multiple comparisons to identify differences between groups and time points.
[a]p<0.001 for longitudinal comparisons of GMT in PAPS patients at D28 and D69 vs. baseline.
[b]p<0.001 for longitudinal comparison of GMT in PAPS patients at D69 vs. D28.
[c]p<0.001 for longitudinal comparison of GMT in control at D28 and D69 vs. baseline.
[d]p<0.001 for longitudinal comparison of GMT in control at D69 vs. D28.

Table 3. Frequency of neutralizing antibodies and neutralizing activity (%) after CoronaVac in näive-PAPS compared to controls.

	After vaccine 1st dose		After vaccine 2nd dose	
	Subjects with positive NAb, n (%)	Neutralizing activity (%) median (interquartile range)	Subjects with positive NAb, n (%)	Neutralizing activity (%) median (interquartile range)
PAPS, *n*=31	5 (16.1)[a]	38.1 (32–55.5)	24 (77.4)	64.3 (49.0–77.0)
Controls, *n*=108	38 (35.2)	43.7 (34.2–66.4)	85 (78.7)	60.9 (45.6–81.3)
p-Value (PAPS vs CG)	*p* =0.043	*p*=0.275	*p*=0.440	*p*=0.689

Results are expressed in median (interquartile range) and n (%).
Nab—neutralizing antibodies; PAPS—primary antiphospholipid syndrome; CG—control group.
Positivity for Nab defined as a neutralizing activity ≥30% (cPass sVNT Kit, GenScript, Piscataway, USA).
[a]p <0.05 in comparison to controls.

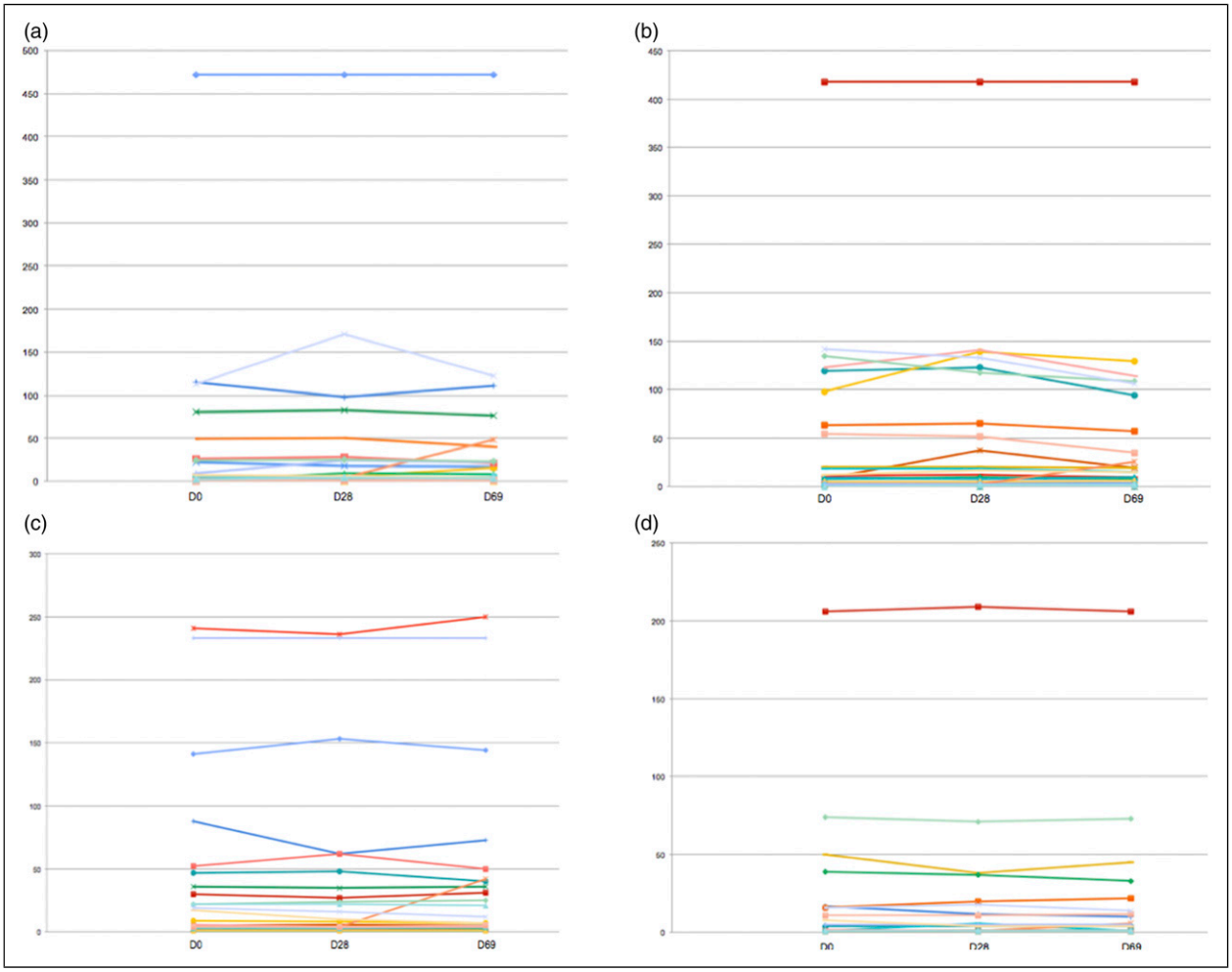

Figure 2. Antiphospholipid antibody titers evaluation in näive primary antiphospholipid patients before (baseline—D0) and after Sinovac-CoronaVac vaccination (first dose—D28 and second dose—D69). (a) Anticardiolipin antibody IgM (aCL, titers in MPL), (b) anticardiolipin antibody IgG (aCL, titers in GPL), (c) anti-beta-2 glycoprotein I IgM (aβ2GPI, titers in UI/mL), and (d) anti-beta-2 glycoprotein I IgG (aβ2GPI, titers in UI/mL).

good safety profile, with no severe AE or underlying disease flare.[30,31] However, lower antibody titers compared to controls were observed, which may impact protection against the virus.[32-33] In line with these findings, our recent study revealed a moderate, but reduced SC rate with Sinovac-CoronaVac in 910 adults with naïve ARD (vs. 182 naïve volunteers in CG). Immunosuppressive drugs and prednisone were identified as factors associated with

Table 4. Adverse events of CoronaVac vaccination in primary antiphospholipid syndrome patients and controls.

	After vaccine 1st dose			After vaccine 2nd dose		
	PAPS (*n*=44)	Controls (*n*=132)	*p*-Value	PAPS (*n*=44)	Controls (*n*=132)	*p*-Value
No symptoms	26 (59.1)	81 (61.4)	0.789	25 (59.5)	86 (66.7)	0.400
Local reactions (at the injection site)	14 (31.8)	27 (20.5)	0.123	8 (19.0)	26 (20.2)	0.876
Pain	12 (27.3)	22 (16.7)	0.123	6 (14.3)	25 (19.4)	0.457
Erythema	5 (11.4)	1 (0.8)	0.004	2 (4.8)	1 (0.8)	0.150
Swelling	2 (4.5)	5 (3.8)	>0.999	3 (7.1)	8 (6.2)	0.732
Bruise	6 (13.6)	4 (3.0)	0.017	0 (0)	1 (0.8)	>0.999
Pruritus	2 (4.5)	1 (0.8)	0.155	2 (4.8)	6 (4.7)	>0.999
Induration	4 (9.1)	6 (4.5)	0.270	2 (4.8)	9 (7.0)	>0.999
Systemic reactions	16 (36.4)	39 (29.5)	0.398	15 (35.7)	37 (28.7)	0.390
Fever	2 (4.5)	2 (1.5)	0.260	2 (4.8)	3 (2.3)	0.597
Malaise	6 (13.6)	5 (3.8)	0.019	3 (7.1)	13 (10.1)	0.764
Somnolence	6 (13.6)	10 (7.6)	0.226	1 (2.4)	9 (7.0)	0.454
Lack of appetite	2 (4.5)	4 (3.0)	0.641	1 (2.4)	6 (4.7)	>0.999
Nausea	6 (13.6)	1 (0.8)	0.001	3 (7.1)	9 (7.0)	>0.999
Vomit	1 (2.3)	1 (0.8)	0.439	0 (0)	1 (0.8)	>0.999
Diarrhea	4 (9.1)	7 (5.3)	0.471	3 (7.1)	7 (5.4)	0.709
Abdominal pain	3 (6.8)	6 (4.5)	0.693	3 (7.1)	7 (5.4)	0.709
Vertigo	5 (11.4)	3 (2.3)	0.024	2 (4.8)	6 (4.7)	>0.999
Tremor	3 (6.8)	0 (0)	0.015	1 (2.4)	2 (1.6)	0.573
Headache	6 (13.6)	16 (12.1)	0.792	8 (19.0)	23 (17.8)	0.859
Fatigue	8 (18.2)	5 (3.8)	0.002	5 (11.9)	14 (10.9)	0.851
Sweating	4 (9.1)	2 (1.5)	0.035	3 (7.1)	3 (2.3)	0.159
Myalgia	4 (9.1)	3 (2.3)	0.067	6 (14.3)	14 (10.9)	0.548
Muscle weakness	5 (11.4)	4 (3.0)	0.044	5 (11.9)	10 (7.8)	0.409
Arthralgia	6 (13.6)	5 (3.8)	0.019	4 (9.5)	9 (7.0)	0.737
Back pain	7 (15.9)	7 (5.3)	0.024	3 (7.1)	16 (12.4)	0.413
Cough	4 (9.1)	4 (3.0)	0.109	0 (0)	6 (4.7)	0.338
Sneezing	4 (9.1)	6 (4.5)	0.270	5 (11.9)	11 (8.5)	0.514
Coryza	3 (6.8)	11 (8.3)	>0.999	5 (11.9)	16 (12.4)	0.932
Stuffy nose	6 (13.6)	6 (4.5)	0.038	2 (4.8)	12 (9.3)	0.522
Sore throat	0 (0)	5 (3.8)	0.333	2 (4.8)	7 (5.4)	>0.999
Shortness of breath	2 (4.5)	4 (3.0)	0.641	0 (0)	4 (3.1)	0.573
Conjunctivitis	0 (0)	0 (0)	-	0 (0)	1 (0.8)	>0.999
Pruritus	2 (4.5)	0 (0)	0.061	2 (4.8)	2 (1.6)	0.253
Skin rash	1 (2.3)	2 (1.5)	>0.999	0 (0)	1 (0.8)	>0.999

Results are presented in n (%). PAPS—primary antiphospholipid syndrome.

diminished immunogenicity evaluating the entire group of ARD patients.[23]

However, PAPS patients may have some distinct clinical and immunological features [34] compared to other ARDs. A previous study published by our group evaluating the response to the H1N1 vaccine in 1668 ARD patients demonstrated that PAPS patients presented higher rates of SC than several other ARDs.[35] The present study with Sinovac-CoronaVac vaccine showed that PAPS patients had a high SC and high NAb positivity, comparable to the CG. The most likely explanation is the fact that the cornerstone of treatment in this syndrome is lifelong anticoagulation and not immunosuppressive therapy.[36] The accuracy of this data was improved by the fact that both groups were balanced by age and sex, one of the most important parameters to influence vaccine response.[37] In addition, the impact of previous exposure in vaccine response was excluded, since only naïve-PAPS patients were evaluated for immunogenicity. In fact, previous studies have demonstrated that vaccine-induced antibody response is greatly enhanced in pre-exposed individuals.[38-39]

The safety profile of inactivated COVID-19 vaccines has been tested and confirmed by mass immunization programs; those vaccines are highly relevant for the population

evaluated in the present study.[40] Our PAPS patients had more minor adverse effects compared to controls. Perhaps the awareness of having a thrombophilia might have alerted them to report any symptom after the first dose. The occurrence of more bruises was expected because of anticoagulation.

Even though the thrombotic risk assessed with aGAPSS in our PAPS patients was very high, no thrombotic event was recorded during our study.[41] In addition, these patients have a high frequency of comorbidities associated with endothelial dysfunction, such as hypertension, obesity, and dyslipidemia, which may also favor clot events.[42-44] Despite the very small sample size, it is reassuring that no cases of venous and arterial thromboses were observed in this high-risk population, after 6 months of follow-up.

Supporting this notion, aPL titers were comparable before and after complete vaccination, an encouraging finding since aPL has an important role in the PAPS thrombogenesis.[10] Consistent with this observation, we have not detected a significant production of aPL-related antibodies nor thrombotic events after the pandemic influenza immunization in PAPS patients.[45] Furthermore, a larger Chinese study with 406 healthy-workers immunized with inactivated SARS-CoV-2 vaccine (BBIBPCorV, Sinopharm, Beijing, China) also found no significant difference in aPL measurement in serial blood samples before and 4 weeks after the second dose.[46]

Our study has some limitations. The routine blood collection used to perform immunogenicity assays of SARS-CoV-2 could not be extrapolated to LA functional assays, a known high-risk parameter for thrombosis in PAPS and perhaps also for COVID-19 infection.[47] Another flaw in our study was the small convenience sample size but very much related to the general prevalence of this disease in the population, which is approximately 50 per 100,000 population,[48] with numbers being even lower when considering only PAPS.

In conclusion, Sinovac-CoronaVac vaccine was highly immunogenic, demonstrated a good safety profile, and did not trigger short- and medium-term thrombosis or production of aPL in naïve-PAPS patients. Our findings support the recommendation of SARS-CoV-2 vaccination for PAPS patients.

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Trial registration

ClinicalTrials.gov- NCT04754698 first registered on February 8th 2021. https://clinicaltrials.gov/ct2/show/NCT04754698?term=NCT04754698&draw=2&rank=1

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