Concise report

COVID-19 vaccine affects neither prothrombotic antibody profile nor thrombosis in primary anti-phospholipid syndrome: a prospective study

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Abstract

Objective. To explore whether inactivated coronavirus disease 2019 vaccine influences the profile of prothrombotic autoantibodies and induces thrombotic events in primary APS patients.

Methods. We enrolled 39 primary APS patients who received two doses of inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine (BBIBPCorV, Sinopharm, Beijing, China) voluntarily in this prospective cohort. Prothrombotic autoantibodies were determined before vaccination and 4 weeks after the second dose of vaccination. Thrombotic disorders were evaluated via hospital site visits and assessments.

Results. There was no significant difference in the presence of all 11 autoantibodies detected before and 4 weeks after vaccination: for aCL, IgG (14 vs 16, $P = 0.64$), IgM (13 vs 19, $P = 0.34$), IgA (2 vs 3, $P = 0.64$); anti-β2GP1, IgG (12 vs 12, $P = 1.00$), IgM (5 vs 8, $P = 0.36$), IgA (4 vs 3, $P = 0.69$); anti-PS/PT IgG (13 vs 16, $P = 0.48$), IgM (17 vs 22, $P = 0.26$); LAC (22 vs 28, $P = 0.16$); aPF4-heparin (0 vs 0, $P = 1.00$) and ANA (23 vs 26, $P = 0.48$). Notably, the distribution of the aPL profile in the pre- and post-vaccination cohorts was not affected by SARS-CoV-2 vaccination: for patients with a low-risk aPL profile (11 vs 10, $P = 0.799$) and patients with a high-risk aPL profile (28 vs 29, $P = 0.799$), respectively. Furthermore, no case exhibited symptoms of the thrombotic disorder during a minimum follow-up period of 12 weeks. There was no adjustment to the ongoing treatment regimens following SARS-CoV-2 vaccination.

Conclusion. Inactivated SARS-CoV-2 vaccine does not influence the profile of anti-phospholipid antibodies and anti-PF4-heparin antibodies nor induces thrombotic events in primary APS patients.

Key words: APS, anti-phospholipid antibody, anti-PF4-heparin antibody, COVID-19 vaccine, thrombosis

CLINICAL SCIENCE

Rheumatology key messages

- The detailed prothrombotic autoantibody profile in pre- and post-vaccinated APS patients remains undepicted.
- This study draws a clinical picture and provides serologic findings in vaccinated primary APS patients.
- This study provides risk-based guidance about the vaccine-receiving tactics of APS patients.

Introduction

The ongoing pandemic of coronavirus disease 2019 (COVID-19), induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a matter of

international concern. While fever and cough are the most common symptoms, clinical evidence is evolving on the long-term effects of COVID-19, which is now recognized as a multi-organ disease. Thromboembolic complication is one of the main causes of death in severe COVID-19

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patients [1], which is potentially associated with the presence of aPL antibodies, including aCL, anti-β2 glycoprotein I (anti-β2GPI), lupus anticoagulant (LAC) and anti-phosphatidylserine/prothrombin (anti-PS/PT) [2, 3]. The presence of aPL antibodies in COVID-19 patients suggests that SARS-CoV-2 may cause abnormal coagulation mediated through some autoimmune mechanism. Furthermore, the coagulopathy and vascular features of critically ill COVID-19 patients shared some similarities with catastrophic APS, which features multi-organic thrombosis [4].

APS is a systemic autoimmune disease characterized by thrombotic or obstetric manifestations with persistent aPL antibodies. As the driving force of APS, aPL antibodies can induce thrombosis through multiple mechanisms [5]. Furthermore, anti-PS/PT autoantibodies are associated with an increased incidence of thrombosis in APS patients.

Certain evidence has confirmed the efficacy of the COVID-19 vaccine to boost immune responses and acquire protection against SARS-CoV-2. Nevertheless, after widespread implementation of the adenovirus vaccine, a considerable number of thrombotic events in vaccine recipients were reported in various organs. Thrombocytopenia and thrombotic events in various sites within weeks after receiving vaccination were initially described in February 2021. An analogous class of severe vaccine-induced adverse effects were named vaccine-induced immune thrombotic thrombocytopenia (VITT), a rare but fatal complication associated with the vaccine against SARS-CoV-2. VITT presents an extensive array of thrombotic events in multiple vascular beds, involving both venous and arterial circulations [6], associated with anti-PF4-heparin antibody positivity. Notably, while VITT does share pathogenic, clinic and serologic similarities with heparin-induced thrombocytopenia (HIT), it predominantly occurs without heparin exposure.

These post-vaccine thrombotic events strongly interfere with the decision to receive vaccine in APS patients. As the presence of anti-PF4 antibodies indicated the autoimmune and hypercoagulable nature of VITT, considering the same characteristic of APS, rheumatologists should be alert to corresponding thrombotic risks. To provide risk-based guidance about the vaccine-receiving tactics of APS patients, as well as management in APS patients who received COVID-19 vaccination, we aim to assess the prothrombotic autoantibody production and thrombotic events as well as the APS-associated clinical picture and serologic findings after COVID-19 vaccination in APS patients.

Patients and methods

Study design and participants

The APS-Shanghai (APS-SH) database was screened and 210 primary APS patients were assessed as eligible. Exclusion criteria included fever, cough and other discomfort symptoms within 14 days before vaccination; thrombotic event or abortion events within 1 year before

vaccination; pregnancy or lactation; or a history of seizures, allergy or mental illness. Among them, 72 patients were subjectively willing to receive inactivated SARS-CoV-2 vaccination while the rest ($n = 138$) were hesitant or unwilling to. However, 21 patients failed to receive the first dose due to exclusion factors—pregnancy ($n = 5$), fever ($n = 6$) or allergy ($n = 10$)—and 12 patients exited the study early due to unwillingness to receive the second dose ($n = 6$) or loss to follow-up ($n = 6$) (Supplementary Fig. S1, available at *Rheumatology* online). A total of 39 primary APS patients who received two doses, 21 days apart, of inactivated SARS-CoV-2 vaccine (BBIBPCorV, Sinopharm, Beijing, China) were voluntarily enrolled in our study. This cohort was recruited from 3 January to 6 February 2022, with a minimum follow-up period of 12 weeks. Prothrombotic autoantibodies were determined 7 days before vaccination and 4 weeks after the second dose of vaccination. Thrombotic disorders were evaluated via hospital site visits and assessments. It is worth noting that all 39 patients were treated normatively according to the EULAR recommendations for the management of APS in adults [7]. Our study obtained approval from the Institutional Review Board of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (approval 2022-81) and written informed consent from participants.

Antibody measurements

Indirect fluorescence on Hep-2 cells was used to assess ANA according to the manufacturer's instructions (Inova Diagnostics, San Diego, CA, USA). An ELISA kit (Quanta Lite, Inova Diagnostics) was used to assess aPL antibodies, including aCL IgG/IgM/IgA, anti-β2GPI IgG/IgM/IgA and anti-PS/PT IgG/IgM according to the manufacturer's instructions. Lupus anticoagulation was determined according to the guidelines of the International Society on Thrombosis and Haemostasis Subcommittee on Lupus Anticoagulant/Phospholipid-dependent Antibodies [8]. An automated latex immunoturbidimetric assay [HemosIL HIT-Ab (PF4-H), Instrumentation Laboratory, Bedford, MA, USA) was used to assess all Ig isotypes of anti-PF4-heparin antibodies. All autoantibodies mentioned above were detected according to the manufacturer's instructions. The suggested cut-off value is 1:80 for ANA according to the international guidelines and ≥20 GPL/MPL/APL for aCL IgG/IgM/IgA, ≥20 SGU/SMU/SAU for anti-β2GPI IgG/IgM/IgA and ≥30 units for anti-PS/PT antibody according to the manufacturer's instructions.

Statistical analysis

SPSS software (version 26.0, IBM, Armonk, NY, USA) was used for the analyses. Data are shown as numbers (percentages) for categorical variables and median [quartile 1 (Q1)–quartile 3 (Q3)] for continuous variables with a skewed distribution. The chi-squared test or Fisher's exact test were used to compare categorical data. A two-sided *P*-value ≤0.05 was considered statistically significant.

Results

Baseline characteristics

Among 72 primary APS patients who satisfied the 2006 Sydney classification criteria from the APS-SH database and were willing to receive SARS-CoV-2 vaccine [9, 10], 39 patients who received two doses of vaccine voluntarily accomplished the whole follow-up process. The cohort's median age was 38 years (Q1–Q3: 29–43) and 82.1% (32/39) were women. Among the cohort, 56.4% (22/39) suffered thrombotic events and 43.6% (17/39) suffered abortion. According to the EULAR recommendation [7], a high-risk aPL profile is defined as the presence of LAC or double/triple aPL positivity or persistently high aPL titres and a low-risk aPL profile is defined as absolute aCL or anti-β2GPI at low–medium titres. The proportion of patients with a low-risk and high-risk aPL profile is 28.2% (11/39) and 71.8% (28/39), respectively. Demographic characteristics of the cohort are shown in Table 1. There was no statistical significance between markers of coagulation activation measured pre- and 4 weeks post-vaccination: APTT [median 42.55 (Q1 31.28–Q3 60.80) vs 43.10 (31.20–53.26), $P = 0.40$], PT [median 15.55 (Q1 11.88–Q3 27.43) vs 12.70 (11.70–21.90)], $P = 0.46$], INR [median 1.92 (Q1 1.53–Q3 2.70) vs 2.04 (1.68–2.31), $P = 0.65$] and D-dimer [median 0.26 (Q1 0.20–Q3 0.32) vs 0.31 (0.26–0.42), $P = 0.14$] (Supplementary Table S1, available at Rheumatology online). All patients had gone through a minimum follow-up period of 8 weeks after receiving the second dose of vaccine. Within 4 weeks after the second vaccination, adverse reactions were reported in 19 (48.72%) study participants. The most common adverse systemic reactions were dizziness and headache (26.92%) and fatigue (26.92%). The most common local adverse reaction was pain and tenderness, reported by 10.26% (4/39). All adverse reactions were mild, transient and self-limiting.

Autoantibody prevalence and the correlation with clinical features

The prevalence of positivity in all 11 autoantibodies determined before and 4 weeks after vaccination were similar: for aCL, IgG (14 vs 16, $P = 0.64$), IgM (13 vs 19, $P = 0.34$), IgA (2 vs 3, $P = 0.64$); anti-β2GP1, IgG (12 vs 12, $P = 1.00$), IgM (5 vs 8, $P = 0.36$), IgA (4 vs 3, $P = 0.69$); anti-PS/PT, IgG (13 vs 16, $P = 0.48$), IgM (17 vs 22, $P = 0.26$); LAC (22 vs 28, $P = 0.16$); aPF4-heparin (0 vs 0, $P = 1.00$) and ANA (23 vs 26, $P = 0.48$) (Supplementary Table S2, available at Rheumatology online). Antibody titres were tested by Wilcoxon rank sum test and the result showed no statistical significance (Supplementary Table S1, available at Rheumatology online). As the aPL profile is an important factor determining the risk of thrombosis and the intensity of treatment, we also focused on the influence of vaccination on the aPL profile. Notably, there was no statistical significance in the distribution of the aPL profile between the pre- and post-vaccinated cohort: for patients with a low-risk

TABLE 1 Clinical and laboratory characteristics of enrolled APS patients before vaccination

| Characteristics	Group enrolment baseline
Age, years, median (Q1–Q3)	38 (29–43)
Sex (female/male), n/n	32/7
Criteria manifestations, n (%)	
Vascular thrombosis	22 (56.4)
Arterial thrombosis	10 (25.6)
Venous thrombosis	12 (30.8)
Pregnancy morbidity	17 (43.6)
Early abortion (<10 weeks)	7 (18.0)
Late abortion (>10 weeks)	10 (25.6)
Non-criteria manifestations, n (%)	
Superficial vein thrombosis	4 (10.3)
Thrombocytopenia[a]	4 (10.3)
Livedo reticularis	3 (7.7)
Migraine	8 (20.5)
Assays of antibodies, n (%)	
aCL IgG	14 (35.9)
aCL IgA	2 (5.1)
aCL IgM	13 (33.3)
Anti-β2GPI IgG	12 (30.8)
Anti-β2GPI IgA	4 (10.3)
Anti-β2GPI IgM	5 (12.8)
LAC	22 (56.4)
Anti-PS/PT IgG	13 (33.3)
Anti-PS/PT IgM	17 (43.6)
ANA	23 (59.0)
Anti-PF4-heparin complex	0 (0)
aPL profile, n (%)	
Low-risk	11 (28.2)
High-risk	28 (71.8)
Medications at baseline, n (%)	
HCQ	36 (92.3)
Warfarin	31 (79.5)
Aspirin	18 (46.2)
Prednisone	1 (2.6)
MMF	3 (7.7)

[a]Thrombocytopenia is defined as a platelet count $<100 \times 10^9$/L.

aPL profile (11 vs 10, $P = 0.799$) and patients with a high-risk aPL profile (28 vs 29, $P = 0.799$), respectively. The change in the autoantibody profiles after inactivated COVID-19 vaccination is presented in Fig. 1. The distribution of all 11 autoantibodies among paired samples are shown in Fig. 1G. Furthermore, no case exhibited symptoms of a thrombotic disorder during a minimum follow-up period of 12 weeks. There was no adjustment to the ongoing treatment regimens following SARS-CoV-2 vaccination.

Discussion

Coronavirus vaccines utilize different viruses or viral parts as immunogen: virus vaccines (weakened or inactivated) use the virus itself, nucleic acid vaccines use RNA

FIG. 1 Changes of autoantibody profiles after inactivated COVID-19 vaccination in APS patients
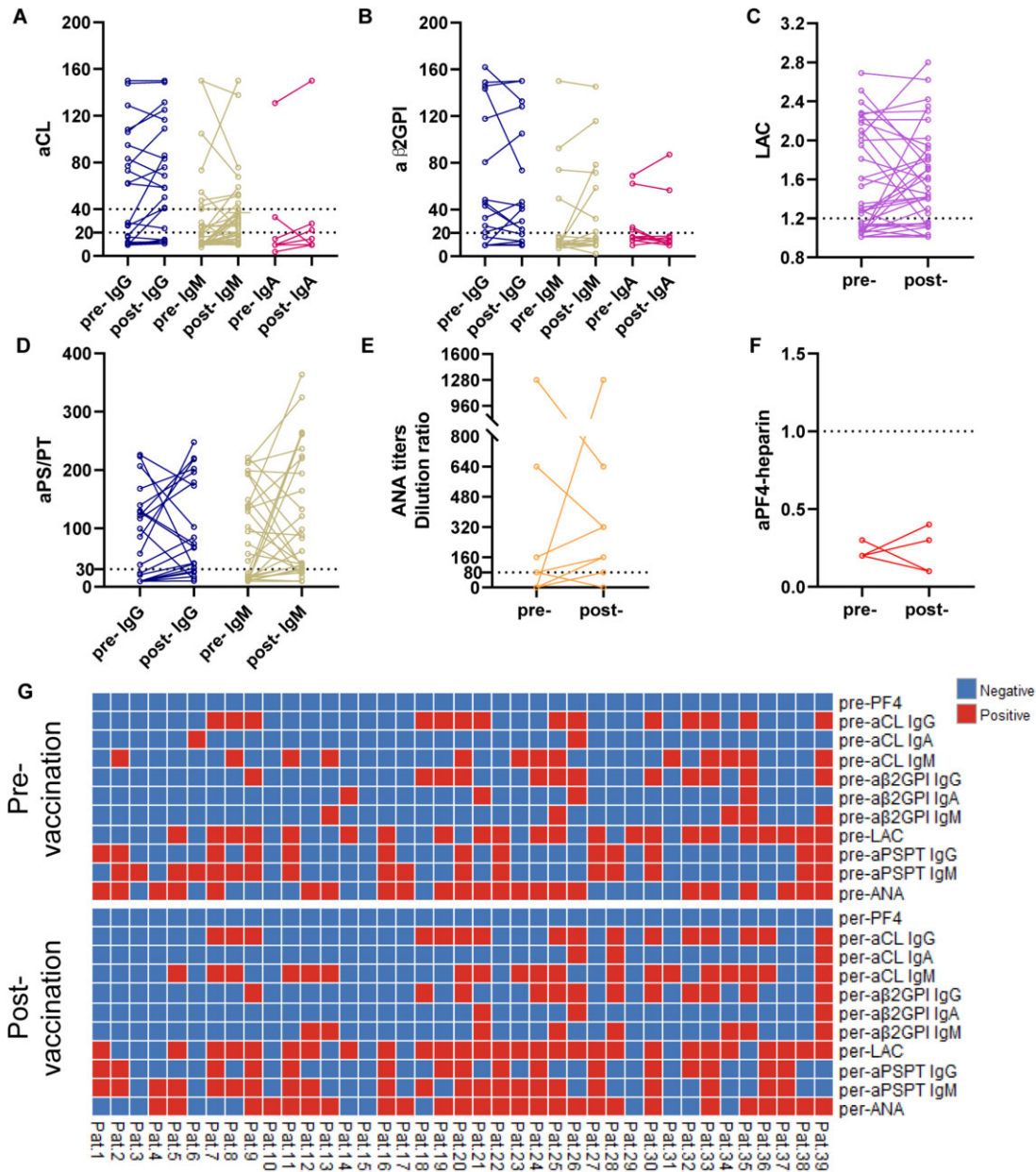
(A) aCL IgG, IgM and IgA; (B) anti-β2GPI IgG, IgM and IgA; (C) LAC; (D) anti-PS/PT IgM; (E) anti-PF4-heparin; (F) ANA titres before vaccination and 4 weeks after the second dose of inactivated SARS-CoV-2 vaccination. The slope of the trend line between pairs indicates the intensity of changes. The horizontal dashed line represents the cut-off value defined by the manufacturers. (G) The heat map presents the distribution of the 11 autoantibodies among paired samples. Pre-: pre-vaccination; post-: post-vaccination (4 weeks after the second dose of inactivated SARS-CoV-2 vaccination).

instructions, weakened measles or adenovirus vaccines produce coronavirus proteins in organism and protein-based vaccines directly inject coronavirus proteins into the body [11]. Nevertheless, they all aim to provoke a specific immune response once injected, implying their mutual possibility of immune thrombosis through some potential mechanism, alerting us to a rare complication. A combination of thrombocytopenia and strongly anti-PF4-heparin

antibody positivity had been reported to precede VITT in some recipients [12]. Cerebral venous thrombosis (CVT) ranked first in VITT, ranging from 38 to 80% in reported cases [13]. The higher mortality rate is related to severe low platelet counts (<30 000/mm³), intracranial haemorrhage and CVT. The clinical and serologic pictures of VITT resemble heparin-induced thrombocytopenia (HIT), featuring thrombosis at unusual sites, thrombocytopenia,

anti-PF4-heparin antibody positivity, elevated D-dimer levels and low fibrinogen levels [13].

The causative mechanism of anti-PF4 antibodies after vaccination is yet to be determined. Cross-reactivity between anti-SARS-CoV-2 and anti-PF4 antibodies was once proposed, but a subsequent study did not support the hypothesis [14]. Anti-PF4 antibodies pathogenically act with FcγRIIA on platelets [15], monocytes and neutrophils to elicit a corresponding hypercoagulable state by procoagulant cellular responses and release of procoagulant microparticles.

The potential role of aPL antibodies in critically ill COVID-19 patients has aroused rheumatologists' concerns, and the protection of COVID-19 vaccine against SARS-CoV-2 infection is urgently needed, especially in the context of the current omicron wave of the COVID-19 pandemic. In our previous prospective study to explore the influence of COVID-19 vaccine on the production of prothrombotic antibodies in healthcare workers in good health, we found that COVID-19 vaccine does not influence the profile of prothrombotic antibodies or increase thrombotic events [16]. However, VITT and APS share a similarity in their thrombogenic and thrombocytopenic natures, leading vaccine-receiving feasibility into a bleak valley. Moreover, the presence of anti-PF4-heparin antibodies and imbedded immune responses engendered corresponding panic in APS patients. A recent study reported anti-PF4 antibodies in aPL-positive patients were not affected by COVID-19 vaccination [17]. Two surveys in patients with aPL antibodies reported that COVID-19 vaccination did not result in severe adverse events [18, 19]. A study reported that COVID-19 vaccine did not affect aCL and anti-β2GP1 IgG and IgM titres in primary APS patients [20]. A step further, our study is the first prospective study designed to evaluate the comprehensive autoimmune antibody profile (including anti-PF4-heparin antibodies) as well as clinical pictures in pre- and post-vaccinated primary APS patients. According to our data, the profiles of aPL antibodies and anti-PF4-heparin antibodies pre- and post-vaccination showed no statistical difference. Notably, no thrombotic event was observed during a minimum follow-up period of 8 weeks after the second dose of inactivated COVID-19 vaccine, compared with VITT observed within 5–30 days after the first dose of the adenovirus-vectored COVID-19 vaccine [13, 17]. Meanwhile, pre- and post-vaccination APS-specific manifestations exhibited non-significant changes. Therefore our study provides more comprehensive insights into the role of inactivated COVID-19 vaccine on primary APS patients. However, we must mention that all 39 participants had taken anticoagulants during the whole process and the influence of anticoagulants on potential thrombotic events cannot be ignored. It is worth noting that there was no statistical significance between markers of coagulation activation measured pre- and 4 weeks post-vaccination.

Some potential limitations of this study need to be mentioned. First, the sample size was limited due to concerns about the influence of COVID-19 vaccination on APS patients. Second, longer-term follow-up is required to further reveal the association between vaccination and prothrombotic autoantibody production.

According to our data, the inactivated vaccine does not increase the risk of prothrombotic antibody-induced thrombotic events in the context of immune system disorders, which provides evidence to rheumatologists for vaccination guidance for APS patients.

Data availability statement

Data are available upon reasonable request to the corresponding author.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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