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Comparison of anti-phospholipid antibody titers before and after SARS-CoV-2 mRNA vaccination in hospital staff

Michi Hisano [a,*], Naho Morisaki [b], Makiko Sampei [b,c], Erika Obikane [b], Koushi Yamaguchi [a]

[a] Center of Maternal-Fetal, Neonatal, and Reproductive Medicine, National Center for Child Health and Development, Tokyo, Japan
[b] Department of Social Medicine, National Center for Child Health and Development, Tokyo, Japan
[c] Department of Nursing and Social Epidemiology, Nippon Sport Science University, Tokyo, Japan

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ABSTRACT

Multiple concerning reports have emerged of cardiovascular complications, particularly thrombosis, following mRNA vaccination against the SARS-CoV-2 pathogen. The presence of serologically persistent anti-phospholipid antibodies is a characteristic of antiphospholipid syndrome, which presents with clinical manifestations including thrombosis or pregnancy morbidity. Anti-SARS-CoV-2 mRNA vaccines pose a theoretical risk of cross-reactivity between the SARS-CoV-2 spike protein and phospholipids in host tissues. In this study, serum anti-phospholipid antibody titers before and after SARS-CoV-2 mRNA vaccination were compared among 184 hospital staff members. Although no significant differences were found in terms of antibody titers targeting cardiolipin and β2-glycoprotein I, post-vaccination antibody titers targeting phosphatidylethanolamine were found to be significantly increased compared to pre-vaccination levels ($p = 0.008$). Anti-phosphatidylethanolamine antibodies are the most common anti-phospholipid antibodies detected in patients with recurrent miscarriages at < 10 weeks of gestation. However, the association between vaccination and these types of adverse events remains unknown, thus warranting further investigation.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared by the World Health Organization (WHO) on March 11, 2020 [1], and exerted a significant impact on global health. Critically ill patients with COVID-19 present with uncontrolled systemic inflammatory responses that may progress to acute respiratory distress syndrome, multiple organ dysfunction, and mortality [2]. Therefore, the timely development of vaccines against SARS-CoV-2 infection was eagerly anticipated. In Japan, the SARS-CoV-2 mRNA vaccines Pfizer-BioNTech Comirnaty® (BNT162b2) and Moderna Spikevax® (mRNA-1273) were approved in February 2021 and May 2021, respectively, as special measures against the outbreak, in accordance with the Pharmaceutical and Medical Device Act. Vaccinations were initiated preferentially for older adults and healthcare workers, who were considered to be at high risk of SARS-CoV-2 infection. The two-dose vaccinations against the original Wuhan strain of SARS-CoV-2 were later expanded to include all persons aged ≥ 12 years, with an initial immunization coverage of ~ 80 %. Booster vaccination doses began to be administered in December 2021, to counteract waning immunity against the disease. With the outbreak of the Omicron variants of SARS-CoV-2, updated vaccines with bivalent Original/BA.1, bivalent Original/BA.4–5 and monovalent XBB.1.5 targeting became sequentially available over 2022–2023. Thus, SARS-CoV-2 mRNA vaccines have been actively administered to many Japanese individuals.

Owing to the limited experience with mRNA-based vaccines, ongoing surveillance is critical to ensuring the long-term safety and efficacy of mRNA-based SARS-CoV-2 vaccines. Multiple reports of cardiovascular complications following SARS-CoV-2 vaccination have raised significant concerns within the medical community [3]. According to a systematic review of original studies reporting confirmed cardiovascular manifestations following SARS-CoV-2 mRNA vaccination [3], thrombosis was the most frequently reported complication following the administration of any mRNA vaccine—followed by stroke, myocarditis, myocardial infarction, pulmonary embolism, and arrhythmia. Thrombosis is also commonly observed in critically ill patients with COVID-19, and is reported to potentially be associated with a

* Corresponding author at: Center of Maternal-Fetal, Neonatal and Reproductive Medicine, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan.
E-mail address: hisano-m@ncchd.go.jp (M. Hisano).

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high prevalence of anti-phospholipid (anti-PL) antibodies in the serum [4–6]. The presence of serologically persistent anti-PL antibodies is characteristic of patients with antiphospholipid syndrome. These patients most commonly present with clinical symptoms including arterial, venous, or microvascular thrombosis; pregnancy-related morbidities; cardiac valve disease; and thrombocytopenia. This study aimed to compare serum anti-PL antibody titers before and after SARS-CoV-2 mRNA vaccination in hospital staff, while concurrently investigating the immunogenicity of these vaccines.

Methods

Study design and participants

Our study was conducted on 184 hospital staff members who participated in both the FY 2020 and FY 2022 wave for a longitudinal study conducted at the Japanese National Center for Child Health and Development during the COVID-19 pandemic, which aimed to assess COVID-19 infection rates as well as mental and physical health among healthcare providers [7]. For this main study, anti-SARS-CoV-2 spike and nucleocapsid IgG antibody titers were measured in March 2021 for FY2020, July 2021 for FY 2021 and July 2022 for FY2022. Surveys on mental and physical health age, sex, job category, height, weight, history of prior SARS-CoV-2 infection (as diagnosed via PCR), total number of SARS-CoV-2 mRNA vaccination doses received, time of most recent vaccination, were administered concurrently. For FY2021 and FY2022 this study was conducted concurrently with routine annual medical check-ups. Among these participants, we chose 221 who had provided consent to use residual serum of the visits at March 2021 and July 2022 for research, and had received at least three doses of a SARS-CoV-2 mRNA vaccine as of July 2022. The exclusion criteria were those who had been previously diagnosed with COVID-19 via PCR or had tested positive for anti-SARS-CoV-2 nucleocapsid IgG antibody. For the 184 remaining participants (Fig. 1), we measured antibody titers to phospholipids, such as cardiolipin (CL), cardiolipin/β2-glycoprotein I (anti-CL/β_2GPI) complex, and phosphatidylethanolamine (PE), using the residual serum. None of the participants had been vaccinated against SARS-CoV-2 at the time of the first blood sampling in March 2021, allowing us to determine changes in anti-PL antibody titers before and after vaccination, as well as the immunogenicity levels of the vaccines.

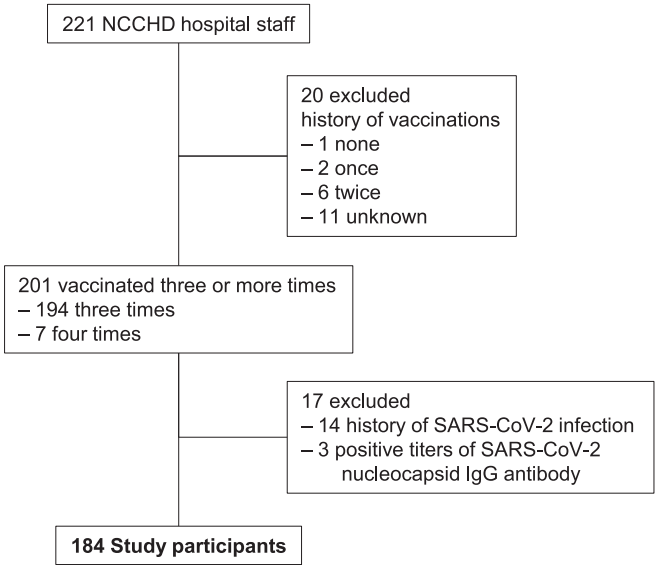

Fig. 1. Flow chart detailing the participant recruitment procedure. A total of 184 participants were drawn from 221 hospital staff who participated in a survey regarding COVID-19 and the measuring of anti-SARS-CoV-2 antibodies at the National Center for Child Health and Development of Japan, in 2021 and 2022. NCCHD: National Center for Child Health and Development.

This study was approved by the local ethics committee of the National Center for Child Health and Development of Japan (Approval No. 2020–266). Written informed consent was obtained from all of the included participants.

Laboratory tests

Anti-SARS-CoV-2 antibody titers were measured using a diagnostic test (Sysmex Corporation, Hyogo, Japan) that detects IgG and IgM specific to SARS-CoV-2 spike and nucleocapsid proteins with high quantifiability and reproducibility, as well as low susceptibility to interference. This system is based on a highly sensitive chemiluminescent enzyme-based immunoassay [8]. To convert antibody titer measurements from antibody units (AUs) to universal binding antibody units (BAUs), a calibration curve was constructed using the First WHO International Standard for anti-SARS-CoV-2 immuno-globulin (human) NIBSC code: 20/136. Serum antibody titers to CL (IgG/IgM) and the CL/β_2GPI complex (IgG) were measured using the commercially available MESACUP^{TM}-2 enzyme-linked immunosorbent assay (ELISA) test (Medical & Biological Laboratories Corporation, Ltd., Tokyo, Japan) and the Yamasa EIA kit (Yamasa Corporation, Tokyo, Japan), respectively.

Serum levels of anti-PE antibody (IgG) were determined using ELISA, according to a previously described procedure [9]. Briefly, microtiter plates were coated with 30 μL of PE (50 μg/mL) diluted in chloroform/methanol mixed solution (1:3) and dried under nitrogen. Each well was blocked for 1 h with 10 % bovine serum albumin in Tris-buffered saline (TBS). PE, the target phospholipid immobilized on the surface of microplate wells, was incubated for 1 h with 50 μL of participant serum diluted (1:100) in TBS containing 10 % adult bovine plasma, followed by alkaline phosphatase-conjugated monoclonal antibody to human IgG. After washing, light absorption of the product formed after adding paranitrophenyl phosphate substrate was measured by optical density at 405 nm. Stop solution with 75 mL of 3 N NaOH was added when the positive controls reached an optical density of 1.0 at 405 nm. Nonspecific binding control wells were processed without phospholipid coating, and the background values were subtracted.

Statistical analysis

A paired Student's *t*-test was used to compare the participants' anti-SARS-CoV-2 and anti-PL antibody titers before and after vaccination. The analyses were done using EZR (Easy R) version 1.65 statistical software, which is based on R and R commander [10]. This software is freely available at: https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html. Statistical significance was set at $p < 0.05$.

Results

Table 1 shows the clinical characteristics of the 184 study participants, most of whom were healthcare workers—including medical doctors, nurses, midwives, and allied health professionals. They had all been vaccinated with Pfizer-BioNTech Comirnaty® (BNT162b2). The number of vaccine doses was three in 177 of the participants, and four in the remaining seven. The period between the last vaccination and the second blood sampling was 0–2 month s in 9 of the participants, 2–4 months in 11, 4–6 months in 5, 6–8 months in 135, 8–10 months in 1, 14–16 months in 5, and unknown in 18. Most of the participants who had received three vaccine doses had a period of 7 months between their last vaccination and the study.

Table 2 shows a comparison of serum antibody titers to phospholipids and SARS-CoV-2 before and after vaccination. No significant difference was found in terms of serum anti-PL antibody titers to CL (IgG/IgM) and the CL/β_2GPI complex (IgG) before and after vaccination. Post-vaccination anti-PE antibody titers (IgG) were significantly increased compared to those prior to vaccination ($p = 0.008$). In addition, the

Table 1
Characteristics of study participants (n = 184).

Variable	Value
Age, median [range] — years	39.5 [24–66]
Sex — no. of subjects (%)	
Male	43 (23)
Female	141 (77)
Job category — no. of subjects (%)	
Medical doctor	45 (24)
Nurse / Midwife	91 (49)
Allied Health professional	13 (7)
Medical administration	13 (7)
General administration	14 (8)
Other	8 (4)
BMI, median [range] — kg/m^2	21.4 [16.1–35.6]
No. of vaccinations — no. of subjects (%)	
Three times	177 (96)
Four times	7 (4)
Period between last vaccination and second blood sampling, median [range] — months	7 [0–16]

BMI: body mass index.

Table 2
Comparison of serum antibody titers to phospholipids and SARS-CoV-2 before and after vaccination.

	Positive threshold	Pre-vaccination	Post-vaccination	*P* value
Anti-phospholipid antibody				
IgG anti-CL, U/mL	12.3	5.5 (3.7)	5.5 (4.2)	0.686
IgM anti-CL, U/mL	20.8	2.6 (3.8)	2.7 (4.1)	0.545
IgG anti-CL/β_2GPI complex, U/mL	3.5	0.4 (1.2)	0.4 (1.8)	0.842
IgG anti-PE, OD_{450nm}	0.300	0.138 (0.071)	0.143 (0.070)	0.009
Anti-SARS-CoV-2 antibody				
IgG anti-Spike, BAU/mL	20	0.6 (0.9)	1134.3 (1331.8)	<0.001
IgG anti-Nucleocapsid, BAU/mL	35	0.3 (0.3)	1.0 (3.6)	0.008

CL: cardiolipin; β_2GPI: β2-glycoprotein I; PE: phosphatidylethanolamine.
Antibody titers are expressed as mean (standard deviation). Each sample was considered to be seropositive if the antibody titer was above the positive threshold.

number of participants who tested positive for IgG anti-PE after vaccination increased from five to nine. However, as shown in the distribution of anti-PE antibody titers before and after vaccination (Fig. 2A), they were within the normal range in 175 participants. In all of the participants, serum antibody titers to the SARS-CoV-2 spike protein increased above the threshold value after vaccination. The distribution of spike protein antibody titers before and after vaccination is shown in Fig. 2B. Although antibody titers to the SARS-CoV-2 nucleocapsid remained below the threshold value after vaccination, post-vaccination antibody titers were significantly higher than those prior to vaccination ($p = 0.009$).

Discussion

In our study on 184 staff members, we observed an increase in anti-PE IgG antibody titers, but not anti-PL antibody titers targeting cardiolipin and β2-glycoprotein I, following administration of a series of BNT162b2 SARS-CoV-2 vaccine. Pfizer-BioNTech Comirnaty® (BNT162b2) is the first mRNA vaccine against SARS-CoV-2 that was introduced in Japan. It consists of a nucleoside-modified mRNA molecule that encodes the stabilized pre-fusion form of the SARS-CoV-2 spike protein encapsulated in a lipid nanoparticle [11]. Similarly to natural SARS-CoV-2 viral infection, transient expression of the full-length spike antigen induces the production of neutralizing antibodies and cellular immune responses. The S1 and S2 subunits of the SARS-CoV-2 spike protein form a phospholipid-like epitope, and one of the mechanisms explaining the development of anti-PL antibodies in COVID-19 is the cross-reactivity between the SARS-CoV-2 spike protein and phospholipids in host tissues [12]. Thus, in theory, vaccination with BNT162b2 could also induce the production of anti-PL antibodies; however, Noureldine et al. reported no association between the administration of the BNT162b2 vaccine and changes in antibody titers specific to phospholipids such as CL, phosphatidic acid, phosphatidylinositol, and phosphatidylserine [13]. Several other studies have also shown limited effects of BNT162b2 vaccination on the frequency of seroconversion for anti-PL antibodies [14–16]. Our results showed that vaccination with BNT162b2 did not affect testing for anti-PL antibodies to CL and the CL/β_2GPI complex, which are included in the diagnostic laboratory criteria for APS. However, serum levels of anti-PE antibodies increased significantly after vaccination, with four of our participants testing positive for seroconversion. PE is a neutrally charged phospholipids that is a major component of cellular membranes across many different organisms. Anti-PE antibodies specifically recognize the kininogen/PE complex rather than PE itself. Kininogen inhibits thrombin-induced platelet aggregation by binding to platelets, and this aggregation has been reported to be enhanced by exogenously added kininogen-dependent IgG anti-PE *in vitro* [17]. Kininogen-dependent anti-PE antibodies may cause thrombosis and are known to be the most common anti-PL antibodies found in patients with recurrent miscarriages at < 10 weeks of gestation [9]. However, this study could not verify whether the increase in anti-PE antibodies is a transient or persistent phenomenon following vaccination, or whether pregnant women are more prone to experiencing adverse effects such as thrombosis and recurrent miscarriages.

Three or four doses of the BNT162b2 vaccine were found to induce sufficient production of anti-SARS-CoV-2 spike protein antibodies. Oosting et al. defined, on the basis of neutralizing capacity, a cutoff level of 300 BAU/mL for anti-SARS-CoV-2 spike protein antibodies to categorize vaccinated individuals as adequate responders (>300 BAU/mL) or suboptimal responders (between > 10 BAU/mL and ≤ 300 BAU/mL) [18]. According to this criterion, the 184 study participants in this study were classified as: 153 (83.2 %) adequate responders, 31 (16.8 %) suboptimal responders, and 0 (0 %) non-responders. None had developed COVID-19 at the time of the routine medical check-up conducted in July 2022. Anti-SARS-CoV-2 nucleocapsid IgG antibody titers were elevated after vaccination, but were found not to surpass the threshold values. This suggests that our participants may have routinely come in contact with patients with various illnesses, including SARS-CoV-2 infections.

Although vaccination with BNT162b2 was found to be highly immunogenic, eliciting high titers of anti-SARS-CoV-2 spike protein antibodies, the long-term safety and efficacy of mRNA-based SARS-CoV-2 vaccines should continue to be scrutinized. The main limitation of this study lies in the fact that it was a retrospective study involving a relatively small cohort of hospital staff.

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CRediT authorship contribution statement

Michi Hisano: Writing – original draft, Data curation. **Naho Morisaki:** Data curation, Writing – review & editing. **Makiko Sampei:** Data curation, Writing – review & editing. **Erika Obikane:** Data curation,

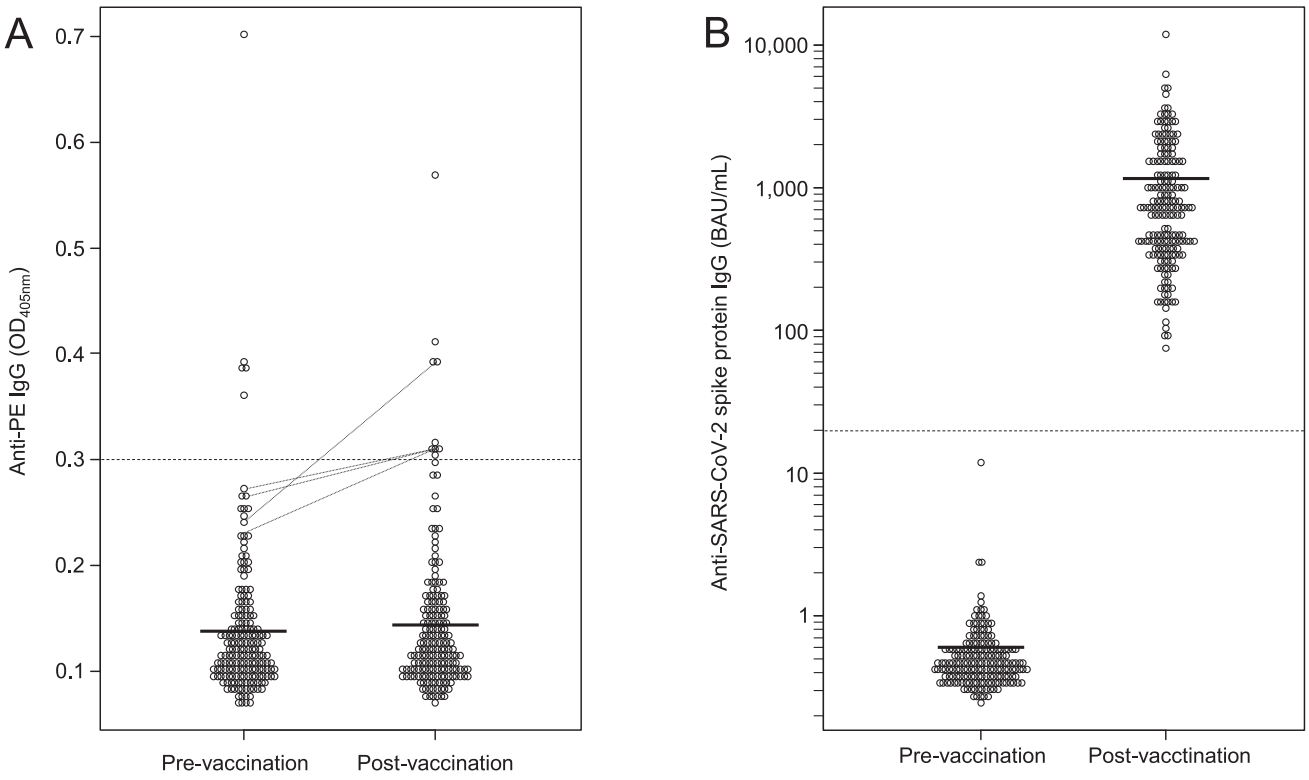

Fig. 2. Distribution of serum antibody titers to the PE and SARS-CoV-2 spike protein before and after vaccination in all 184 study participants. **(A)** Post-vaccination anti-PE IgG antibody titers were significantly increased compared to those prior to vaccination ($p = 0.008$). The lines between the dots represent the change in antibody titers in seroconverted participants. The horizontal dashed line indicates the threshold value to be considered positive (>0.3 OD_{405nm}). The horizontal solid lines indicate the mean values. **(B)** Elevated antibody titers above the threshold were observed in all of the participants following vaccination. The horizontal dashed line indicates the threshold value to be considered positive (>20 BAU/mL). The horizontal solid lines indicate the mean values. PE: phosphatidylethanolamine.

Writing – review & editing. **Koushi Yamaguchi:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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