Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Journal of Clinical Virology 150-151 (2022) 105156

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Immunogenicity and safety of homologous and heterologous ChAdOx1-S and mRNA-1273 vaccinations in healthy adults in Taiwan

Chun-Min Kang [a,b], Nan-Yao Lee [c], Chih-Hsueh Lin [d], Yuan-Shan Hsu [e], Yu-Chang Chang [f], Ming-Yi Chung [a], Ya-Fan Lee [a], Wen-Pin Tseng [g], Jhong-Lin Wu [g], Shey-Ying Chen [g,h,*], Min-Chi Lu [i,j,**], Wen-Chien Ko [c,***], Ping-Ing Lee [b,****], Po-Ren Hsueh [a,f,k,l,*****]

[a] Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan,
[b] Department of Pediatrics, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan
[c] Department of Medicine, College of Medicine, National Cheng Kung University; Department of Internal Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[d] Division of family medicine, China Medical University Hospital, China Medical University, Taichung, Taiwan
[e] Nursing Department, China Medical University Hospital, China Medical University, Taichung, Taiwan
[f] Department of Laboratory Medicine, China Medical University Hospital, China Medical University, Taichung, Taiwan
[g] Department of Emergency Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan
[h] Center for Quality Management, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan,
[i] Department of Microbiology and Immunology, School of Medicine, China Medical University, Taichung, Taiwan
[j] Division of Infectious Diseases, Department of Internal Medicine, China Medical University Hospital, China Medical University, Taichung, Taiwan
[k] Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan,
[l] Ph.D Program for Aging, School of Medicine, China Medical University, Taichung, Taiwan

ARTICLE INFO

Keywords:
COVID-19 vaccines
Safety
Immunogenicity
Homologous and heterologous prime-boost regimens

ABSTRACT

Background: : In Taiwan, the vaccination program started in March 2021, with ChAdOx1-S being the first available WHO-approved COVID-19 vaccine, followed by Moderna vaccine. This study aimed to investigate the immunogenicity and safety of homologous and heterologous prime-boost regimens with ChAdOx1-S and mRNA-1273.

Methods: : From March to November 2021, homologous or heterologous regimens with ChAdOx1-S and mRNA-1273 vaccination (ChAdOx1-S/ChAdOx1-S, mRNA-1273/mRNA-1273, ChAdOx1-S/mRNA-1273) were given to 945 healthy participants. Serum samples were collected at designated time points. The anti-RBD/S1 antibody titers and neutralizing ability were measured by three different immunoassays: Elecsys® Anti-SARS-CoV-2 S (Roche Diagnostics, Mannheim, Germany), AdviseDx SARS-CoV-2 IgG II (Abbott Diagnostics Division, Sligo, Ireland), and cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript, New Jersey, USA).

Results: : We found that heterologous vaccination with ChAdOx1-S/mRNA-1273 had an acceptable safety profile and induced higher total anti-RBD/S1 antibody production ($p < 0.0001$), yet lower anti-RBD/S1 IgG titer ($p < 0.0001$) and neutralizing ability ($p = 0.0101$) than mRNA-1273/mRNA-1273 group. Both regimens showed higher antibody titers and superior neutralizing abilities than ChAdOx1-S/ChAdOx1-S. An age-dependent antibody response to ChAdOx1-S/mRNA-1273 was shown after both the priming and the booster doses. Younger age was associated with higher antibody production and neutralizing ability.

* Corresponding author. Department of Emergency Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, 7, Chung Shan South Road, Taipei 100, Taiwan.
** Corresponding author. Department of Microbiology and Immunology, School of Medicine, China Medical University, Taichung, Taiwan.
*** Corresponding author. Department of Internal Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan.
**** Corresponding author. Department of Pediatrics, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan.
***** Corresponding author. Departments of Laboratory Medicine and Internal Medicine, China Medical University Hospital, China Medical University, No. 2, Yude Road, North District, Taichung 40447, Taiwan.
E-mail addresses: erdrcsy@gmail.com (S.-Y. Chen), luminchi@hotmail.com (M.-C. Lu), winston3415@gmail.com (W.-C. Ko), pinging@ntu.edu.tw (P.-I. Lee), hsporen@ntu.edu.tw (P.-R. Hsueh).

https://doi.org/10.1016/j.jcv.2022.105156
Received 12 February 2022; Received in revised form 13 March 2022;
Available online 6 April 2022
1386-6532/© 2022 Elsevier B.V. All rights reserved.

Conclusions: : Heterologous ChAdOx1-S/mRNA-1273 vaccination regimen is generally safe and induces a robust humoral immune response that is non-inferior to that of mRNA-1273/mRNA-1273.

1. Introduction

The devastating coronavirus disease 2019 (COVID-19) outbreak, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global threat that has resulted in over 5000,000 mortalities [1,2]. The capricious nature of the virus, with increasing numbers of variants, posed great challenges to the healthcare system and caused detrimental effects to economy and social life [3,4]. To overcome the pandemic, vaccines were developed and manufactured using novel techniques. At the time of writing this article, three World Health Organization (WHO)-approved vaccines were available in Taiwan, including ChAdOx1-S (AstraZeneca, Oxford, UK), mRNA-1273 (ModeRNA, Cambridge, MA USA, hereafter referred to as mRNA-1273 or Moderna vaccine), and BNT162b2 (BioNTech Manufacturing, ComiRNAty, BioNTech, Mainz, Germany, hereafter referred to as BNT vaccine) [5].

As an area with low COVID-19 prevalence, vaccine acceptance was relatively low at the beginning because of vaccine hesitancy [6–8].

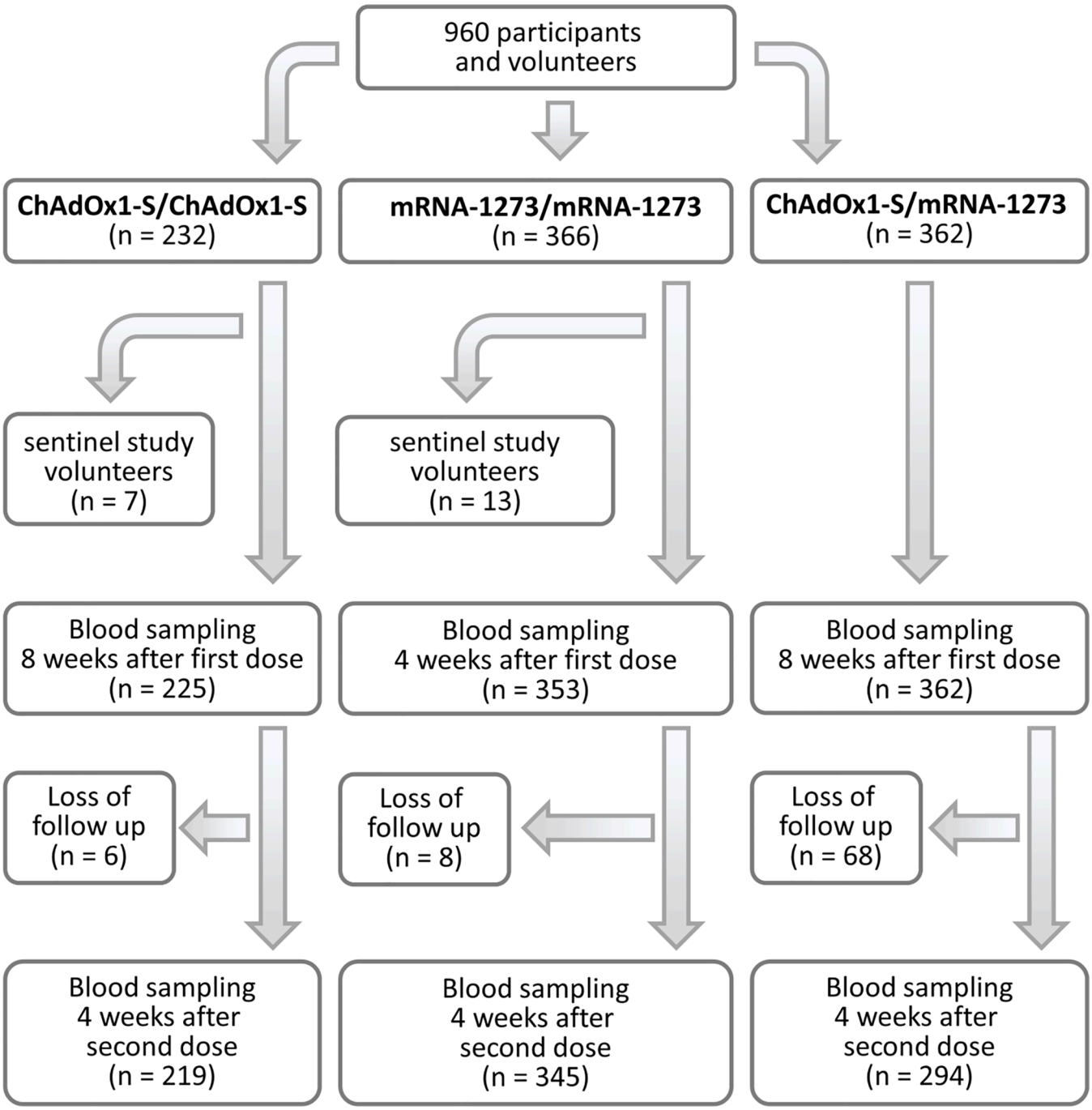

Fig. 1. Flow of participants and volunteers through the study.

However, an outbreak in mid-2021 urged the need for immunization. Heterologous vaccination program was thus been considered with possible benefits of supply chain flexibility and avoidance of serious adverse effects [9–12]. In this study, we aimed to evaluate the immunogenicity and safety of heterologous vaccines with the prime-boost sequence of ChAdOx1-S/mRNA-1273, which were compared with those of homologous regimens of ChAdOx1-S/ChAdOx1-S and mRNA-1273/mRNA-1273. Both ChAdOx1-S and mRNA-1273 have well-established safety profile data and evidence of clinical efficacy [13, 14]. Multiple platforms including several antibody-detecting immunoassays, one competitive enzyme-linked immunosorbent assay (ELISA) and one neutralizing assay were used to evaluate humoral immune responses.

2. Methods

2.1. Study design and enrollment of participants

This was a prospective and multiple-center study. We enrolled healthy participants in this study conducted at the National Taiwan University (NTUH), China Medical University Hospital (CMUH), and National Cheng Kung University Hospital (NCKUH) from May 2021 (Fig. 1). Each participant was subjected to venipuncture up to three times: right before the first dose (Day 0, immediately before the second dose (V2), and four weeks after the second dose (V4) (Fig. 2). The participants were categorized into one of the following three vaccination programs depending on the availability of vaccines at the time of enrollment: ChAdOx1-S/ChAdOx1-S, with an eight-week interval between doses; mRNA-1273/mRNA-1273, with a four-week interval; or a heterologous prime-boost combination of ChAdOx1-S/mRNA-1273, with an eight-week interval.

Twenty volunteers formed the sentinel group to evaluate the performance of immunoassays and weekly changes of antibody titers. Seven of them received ChAdOx1-S/ChAdOx1-S vaccination and thirteen received mRNA-1273/mRNA-1273. Volunteers in the sentinel study were subjected to weekly venipuncture from Day 0 to V4 (Fig. 2). Patient characteristics were collected on the day of enrollment (Table 2). The study was approved by the institutional review board of the NTUH (202101064RINB), CMUH (CMUH110-REC1–090), and NCKUH (A-BR-110–029), and informed consent was obtained from all participants.

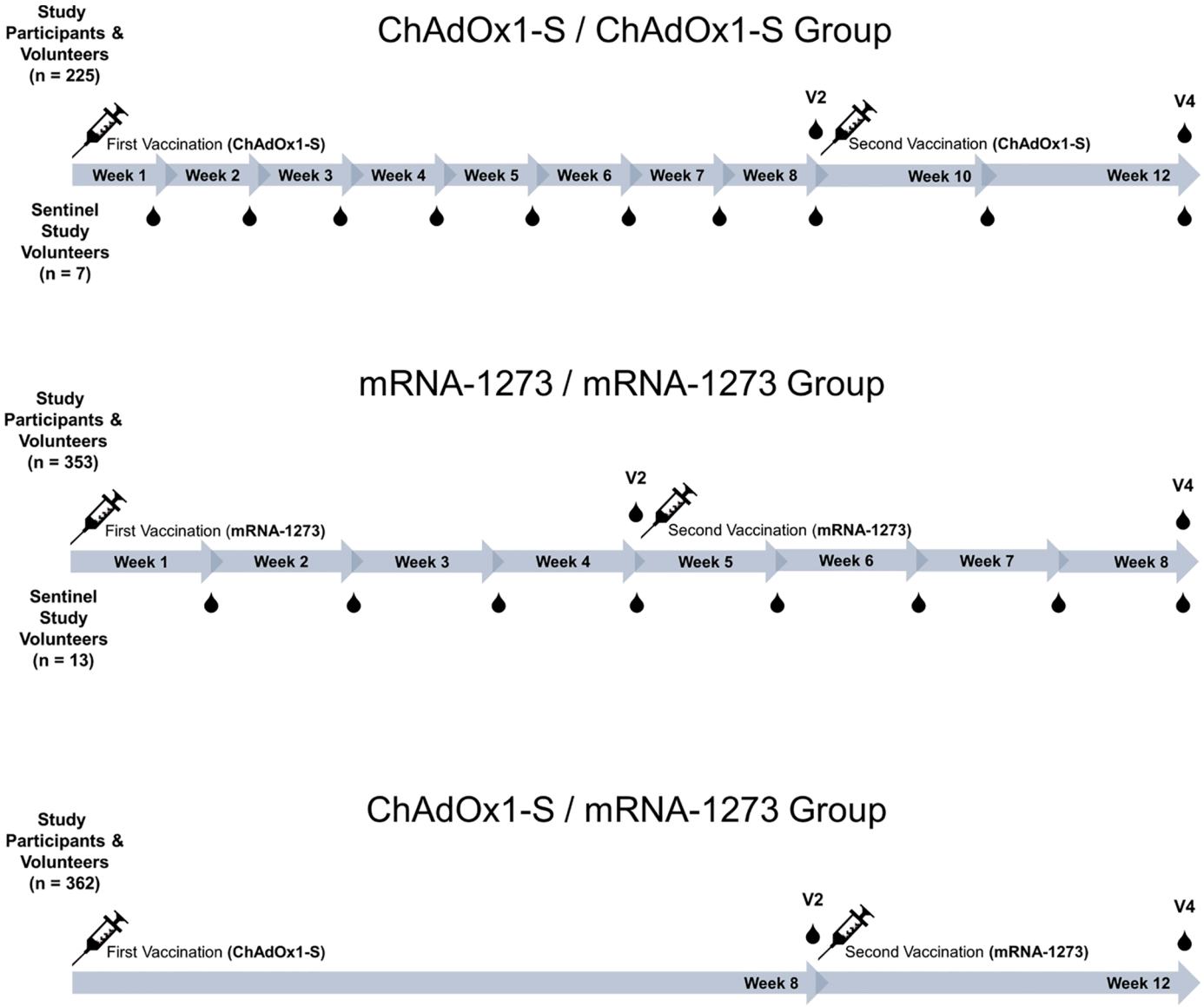

Fig. 2. Timeline of blood sampling.

2.2. Safety evaluation

All study participants were asked to complete an online health questionnaire to report local and systemic adverse events within seven days after the first and second dose. The participants were allowed to report severe or unbearable symptoms directly to the research members at any time during the study, with timely response from the medical professionals.

2.3. Immunoassays and neutralization assays

All blood samples were collected using anti-coagulant-free serum-separating blood tubes. After venipuncture, the tubes were centrifuged, and collected sera were stored at no higher than −20 °C if not tested immediately. Seven automated immunoassays, a competitive enzyme-linked immunosorbent assay (ELISA) and a microneutralization assay were used for measure antibody responses. Details of the assays are presented in Table 1 and the Supplementary Materials.

2.4. Statistical analysis

All data were analyzed using STATA software. Nonparametric tests (Mann–Whitney test and Kruskal–Wallis test) were used to compare antibody levels between different groups. Correlation analysis between assays was done with Pearson's correlation, and statistical significance was set at p-values less than 0.05. Univariate logistic regression was used for the analysis between quantitative and semi-quantitative measures and plotting of receiver operating characteristic (ROC) curves.

Table 2
Basic demographic data of all participants.

Regimen	ChAdOx1-S/ ChAdOx1-S	mRNA-273/ mRNA-1273	ChAdOx1-S/ mRNA-1273
Sample size (n)	225	353	367
Female (%)	56.89	47.59	53.13
Age (years), mean (95% CI)	49.52 (47.25–51.79)	53.71 (52.16–55.26)	52.04 (50.46–53.62)

CI, confidence interval.

3. Results

3.1. Participants and samples

From March to October 2021, blood samples were obtained from 20 volunteers in the sentinel study (7 for ChAdOx1-S/ChAdOx1-S and 13 for mRNA-1273/mRNA-1273), and 945 participants (225 for ChAdOx1-S/ChAdOx1-S, 353 for mRNA-1273/mRNA-1273, and 367 for ChAdOx1-S/mRNA-1273). The mean age of mRNA-1273/mRNA-1273

Table 1
The immunoassays used for evaluating the antibody response after the first dose of the ChAdOx1 COVID-19 vaccine.

Kit	Elecsys Anti-SARS-CoV-2 S	ACCESS SARS-CoV-2 II IgG	ACCESS SARS-CoV-2 IgG 1st IS	ADVIA Centaur® SARS-CoV-2 IgG (sCOVG) assay	AdviseDX SARS-CoV-2 IgG II	EliA SARS-CoV-2-Sp1 IgG P2 Research	EliASARS-CoV-2-Sp1 IgM P2 Research
Company (city, country)	Roche Diagnostics GmbH (Mannheim, Germany)	Beckman Coulter Diagnostics, Inc. (Brea, USA)		Siemens Healthcare Diagnostics Inc. (Tarrytown, USA)	Abbott Ireland Diagnostics Division (Sligo, Ireland)	Thermo Fisher Scientific, Inc. (MA, USA)	
Targeting antibody	high affinity antibodies (IgG included)	IgG		IgG	IgG	IgG	IgM
Immunoassay	ECLIA	CLIA		CLIA	CMIA	FEIA	
Analyzer	Cobas e411, e601 & e602	Access 2 Immunoassay System analyzer		Atellica® IM Analyzer	Architect i system	Phadia 250	
Protein targeting	Recombinant RBD of S1 protein	Recombinant RBD of S1 protein		Biotinylated S1 RBD antigen	Purified SARS-CoV-2 recombinant antigen	Recombinant S1 protein	
Specimen; amount required	Serum or plasma 20 μL	Serum or plasma 20 μL		Serum or plasma 40 μL	Serum or plasma 25 μL	Serum or plasma 20 μL	
Unit conversion	1 U/ml = 0.972 BAU/ml	1 IU/ml = 1 BAU/ml		1 index = 21.8 BAU/ml	1 AU/ml = 0.142 BAU/ml	NA	
Positive result cutoffs and units	≥ 0.80 U/mL	≥ 10 AU/mL	≥ 10 IU/mL	≥ 1.00 Index (U/ml)	≥ 50.0 AU/mL	> 10 U/ml (7–10 equivocal)	
Testing time	18 min	NA		NA	NA	1 min interval after first test	
Reported best sensitivity/ PPA (95% C.I.)	98.8% (98.1–99.3%)	98.9% (92.7–100%)	100% (91.4–100%)	96.41% (92.74–98.54%)	Functional sensitivity.	100% (85.8–100%)	NA
Timing of best sensitivity	–	15–60 days	≥ 14 days	≥ 21 days	does not apply	> 8 days	–
Reported best specificity/ NPA (95% C.I.)	99.98% (99.91–100%)	99.9% (99.5–100%)	99.8% (99.4–99.9%)	99.90% (99.64–99.99%)	Not addressed	100% (99.5–100%)	NA
Confirmed cross-reactivity							
Non-coronaviruses	None	None	None	None	None	None	None
Other seasonal coronaviruses	No cross reactivity with antibodies to MERS-CoV and Common Coronavirus panel	May cause positive results	No cross reactivity to 229E, NL63, HKU1 or OC43	No cross reactivity with antibodies against 29 human coronaviruses	No cross reactivity with antibodies against 229E, HKU1, NL63, OC43	No cross reactivity with antibodies against 229E, HKU1, NL63, OC43	NA

BAU, binding antibody units; CLIA, chemiluminescent immunoassay; CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminescence immunoassay; FEIA, fluorescence enzyme immunoassay; LFA, lateral flow assay; MERS, Middle East respiratory syndrome; NPA, negative percent agreement; NTD, N-terminal domain; PPA, positive percent agreement; RBD, receptor-binding domain.

group was significantly higher than that of ChAdOx1-S/ChAdOx1-S group, mainly because more elderly individuals received mRNA-1273 vaccination under the government's priority policy. There was no age difference between other groups.

3.2. Safety evaluation

Approximately 95.43% of all participants experienced mild to moderate adverse events within seven days after vaccination. The most reported adverse events include tenderness at the injection site, fatigue, muscle soreness, fever, and headache (Fig. 3). The event rates for the prime/booster dose were 84.82%/71.65% for ChAdOx1-S/ChAdOx1-S, 82.75%/89.34% for mRNA-1273/mRNA-1273, and 70.46%/98.54% for ChAdOx1-S /mRNA-1273 group. No serious or life-threatening adverse events were reported in the present study.

3.3. Sentinel study

The weekly changes of antibody titers from 20 volunteers are shown in Fig. 4 (ChAdOx1-S/ChAdOx1-S) and Fig. 5 (mRNA-1273/mRNA-1273). Almost all volunteers had an exponential surge in anti-S1/RBD antibody titers after each vaccination, followed by a gradual decrease in the slope. The only exception was the Thermo Fisher IgM assay, in which the levels of anti-RBD/S1 IgM antibodies increased and declined immediately after the first dose of ChAdOx1-S for most volunteers. Most samples taken after vaccination showed a positive neutralizing ability using the GenScript cPass™ assay (≥ 30% inhibition), while the mRNA-1273/mRNA-1273 regimen seemed to induce stronger neutralization effect than ChAdOx1-S/ChAdOx1-S.

3.4. Correlation analysis

The correlation of measures between different immunoassays is shown in Supplemental Fig. 1. The correlation between IgG-measuring assays (Abbott, both Beckman assays, Siemens, and Thermo Fisher) was good, with coefficients higher than 0.7. The correlation between the Roche kit and the other kits varied. On the other hand, the correlation coefficients between $TCID_{50}$ and other assays were generally low, with the GeneScript cPass™ assay being the highest ($R = 0.6358$). The results are shown in the Supplementary Materials. Univariate logistic regression and ROC curves were plotted using GeneScript cPass™ as the reference method (cutoff: 30%). Most of the immunoassays had optimal predictive values (area under the curve (AUC) > 0.9), except for the Thermo Fisher IgM assay (Supplemental Fig. 2). Based on the performance and the accessibility for operation of different assays, three assays were chosen for completing subsequent analysis, including the Roche, the Abbott and the GeneScript cPass™ assays.

3.5. Screening for previously occult SARS-CoV-2 infection

All samples collected from participants at Day 0 and V2 visits were negative for anti-N antibody (< 1.0 cutoff index [COI]), except the

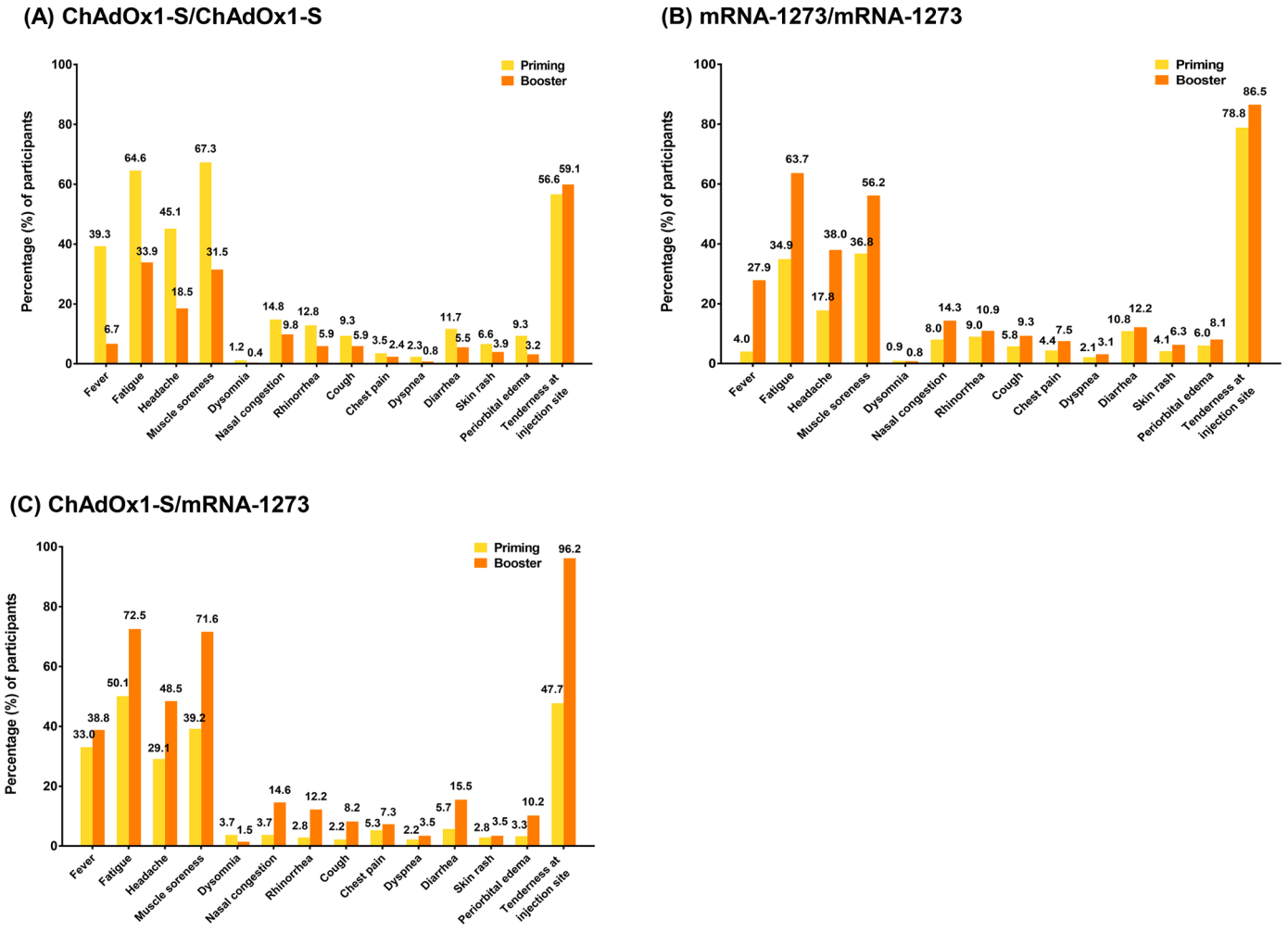

Fig. 3. Percentage of reported adverse events in the seven days following each dose of vaccine injection. (A) ChAdOx1-S/ChAdOx1-S, (B) mRNA-1273/mRNA-1273, (C) ChAdOx1-S/mRNA-1273.

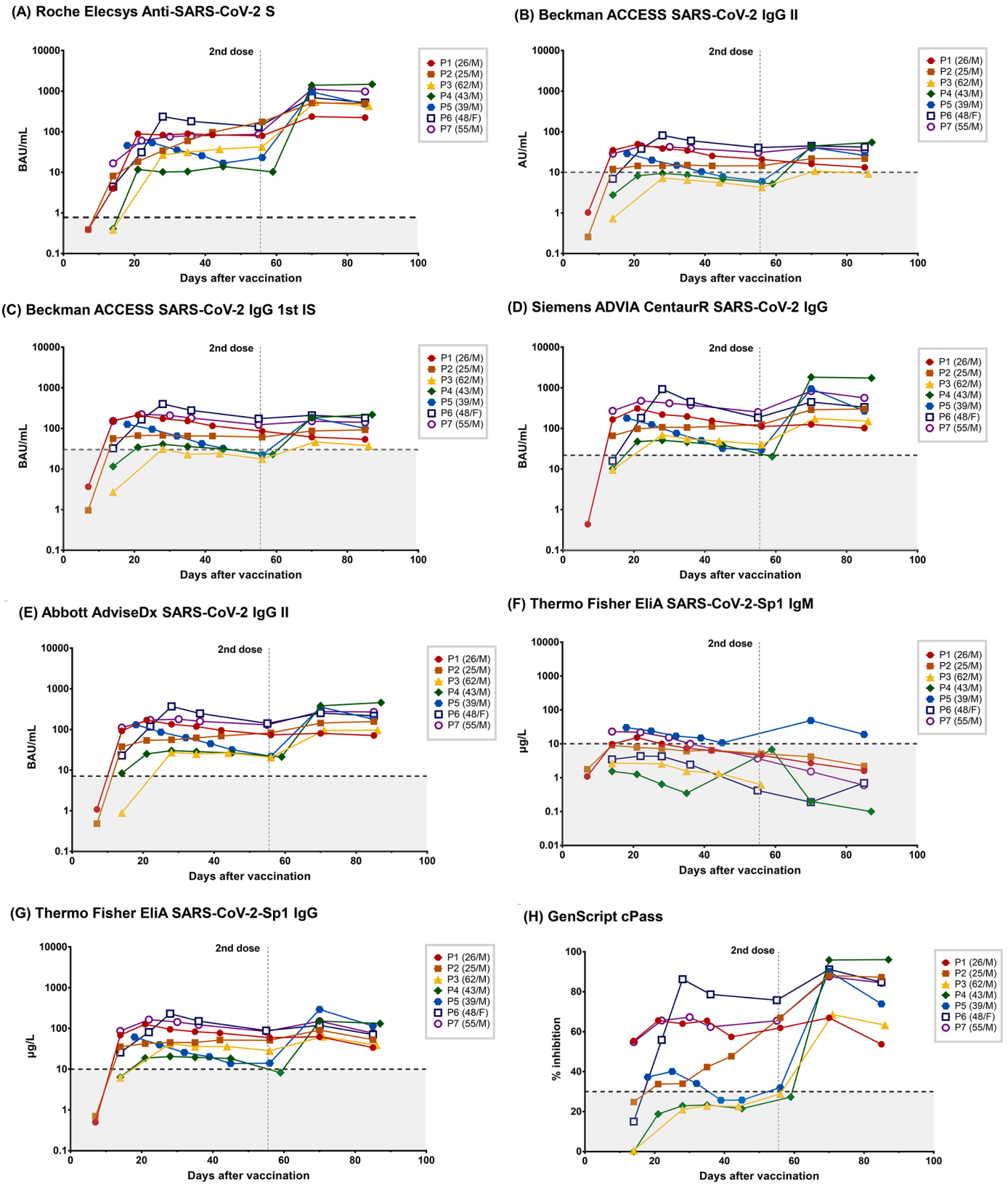

Fig. 4. Individual trends in anti-RBD/S1 antibody levels in 7 sentinel study volunteers receiving ChAdOx1-S/ChAdOx1-S regimen, plotted with logarithmic vertical axis. The vertical dotted line denotes the duration of the second vaccination. The horizontal dashed line with gray rectangle shade indicates the cutoff value of each assay according to the respective package inserts. (A) Roche Elecsys® Anti-SARS-CoV-2 S, (B) Beckman Coulter ACCESS SARS-CoV-2 II IgG, (C) Beckman Coulter ACCESS SARS-CoV-2 IgG 1st IS, (D) Siemens ADVIA Centaur® SARS-CoV-2 IgG (sCOVG) assay; (E) Abbott AdviseDX SARS-CoV-2 IgG II; (F) Thermo Fisher EliA SARS-CoV-2-Sp1 IgG P2 Research, (G) Thermo Fisher EliA SARS-CoV-2-Sp1 IgM P2 Research.

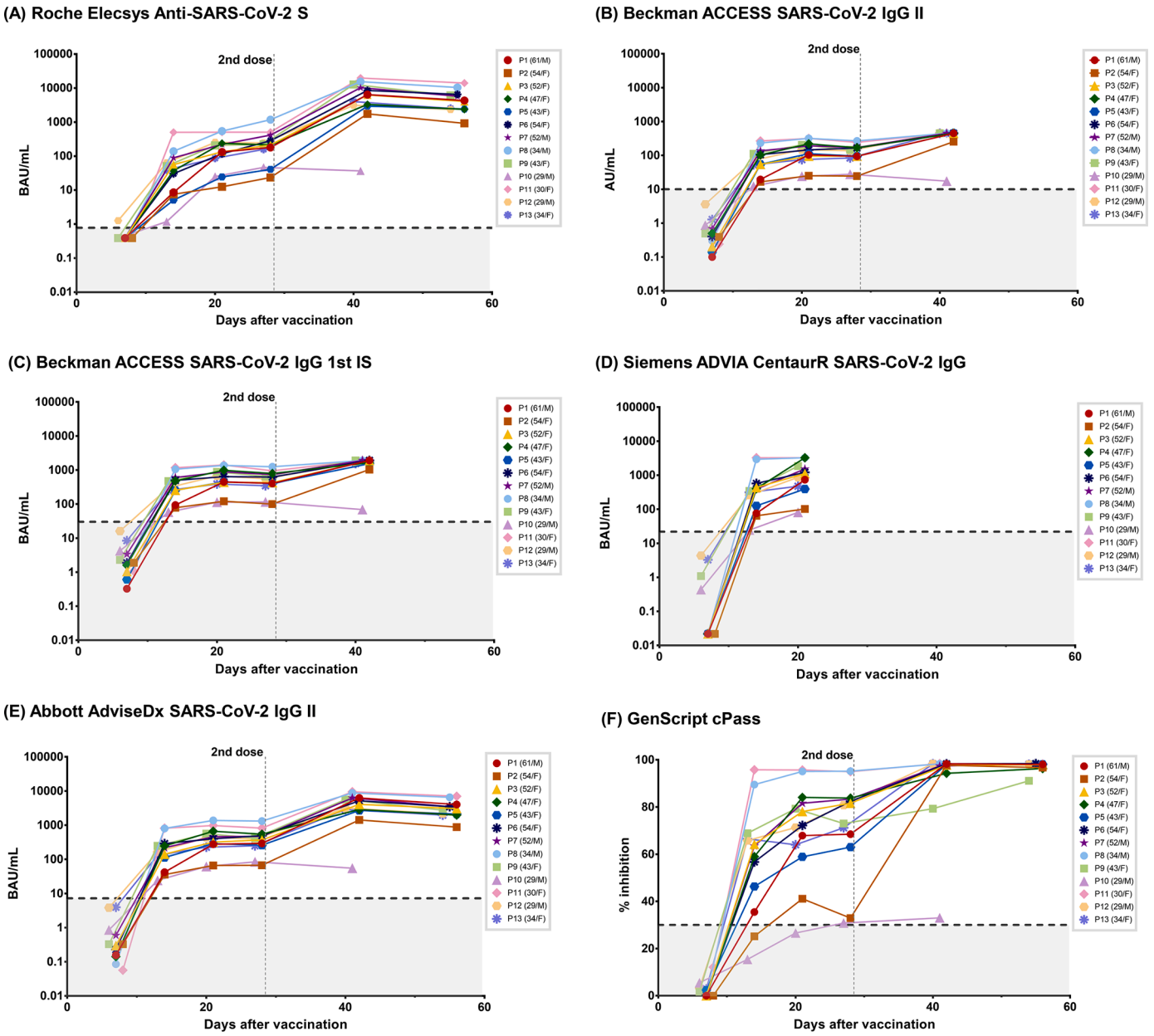

Fig. 5. Individual trends in anti-RBD/S1 antibodies in 13 sentinel study volunteers receiving mRNA-1273/mRNA-1273 regimen, plotted with logarithmic vertical axis. The vertical dotted line denotes the duration of the second vaccination. The horizontal dashed line with gray rectangle shade indicates the cutoff value of each assay according to the respective package inserts. (A) Roche Elecsys® Anti-SARS-CoV-2 S, (B) Beckman Coulter ACCESS SARS-CoV-2 II IgG, (C) Beckman Coulter ACCESS SARS-CoV-2 IgG 1st IS, (D) Siemens ADVIA Centaur® SARS-CoV-2 IgG (sCOVG) assay, (E) Abbott AdviseDX SARS-CoV-2 IgG II.

samples from two participants. The samples were consistent positive at low levels at both Day 0 and V2. The results were judged to be false positive reactions because both participants had no detectable anti-S protein antibody at Day 0 [15,16]. These findings indicated no evidence of occult and unidentified SARS-CoV-2 infections among the study participants before entering the study and before V2.

3.6. Antibody responses of different vaccine regimens

The analysis of antibody response was done using three assays: the Roche and the Abbott assays for measuring antibody titers and GeneScript cPass™ for evaluation of neutralizing ability. For Roche assay, mRNA-1273 generated significantly higher titers of antibodies at V2 than those receiving ChAdOx1-S as first dose. At V4, the heterologous vaccines (ChAdOx1-S/mRNA-1273) generated the highest titer, followed by mRNA-1273/mRNA-1273 with ChAdOx1-S/ChAdOx1-S being the lowest of the three (Fig. 6). The results of V2 samples measured by the Abbott assay showed similar trends as those by Roche assay. However, for V4 samples, mRNA-1273/mRNA-1273 vaccination generated the highest anti-RBD/S1 IgG titers, followed by ChAdOx1-S/mRNA-1273 group, and the difference was statistically significant. The antibodies generated by mRNA-1273 vaccination seemed to have a dominant effect on the neutralizing ability, compared with that by ChAdOx1-S vaccination.

3.7. Antibody response in different age groups

The participants were divided into three age groups: 20–40 years old, >40–60 years old, and >60 years. For V2 samples, antibody production and neutralizing ability were inversely correlated with age (p-values $<$ 0.05 for all regimens), implying a better immune response in younger people after the first dose of vaccine. For V4, however, the samples of

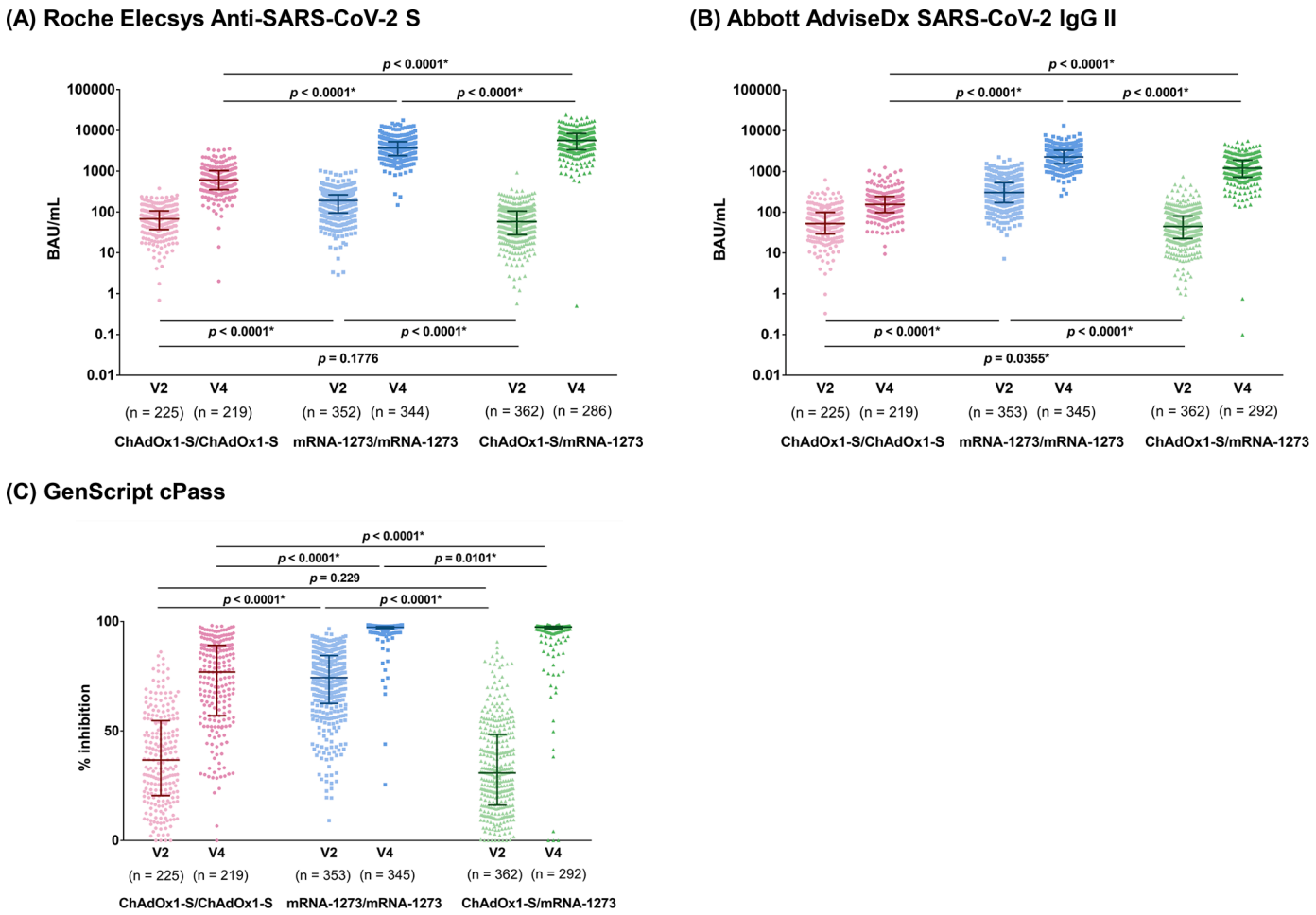

Fig. 6. Antibody titers of all participants and volunteers with different vaccination regimens. The whiskers denote the median (long) and the first and third interquadrant (short) values of all measurements. The analysis was done using Mann-Whitney U test. The vertical axes of Fig. 4(A) and Fig. 4(B) are scaled in a logarithmic manner. (A) Measurements by Roche Elecsys® Anti-SARS-CoV-2 S, (B) Measurements by Abbott AdviseDX SARS-CoV-2 IgG II, (C) Measurements by GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit.

ChAdOx1-S/ChAdOx1-S group showed an opposite trend compared with that of V2 by anti-RBD/S1 antibody measured by the Roche assay. In another word, older age generated higher serum levels of anti-RBD/S1 antibody after booster dose ($p = 0.0129$). However, this V4 trend was not seen in the Abbott assay ($p = 0.4447$), nor in the GeneScript cPass™ assay ($p = 0.5969$). As for the V4 samples from the mRNA-1273/mRNA-1273 group, no age-related variation was found in terms of antibody production or neutralizing ability. In contrast, the V4 samples of ChAdOx1-S/mRNA-1273 group showed an inverse correlation of antibody levels to age using all three assays ($p = 0.0112$, 0.0228, and 0.0264 for the Roche, Abbot, and GeneScript cPass™ assays, respectively) (Fig. 7).

4. Discussion

In this study, we evaluated the antibody responses and safety profiles of the heterologous vaccine regimen of ChAdOx1-S/mRNA-1273 in addition to ChAdOx1-S/ChAdOx1-S and mRNA-1273/mRNA-1273 homologous regimens in Taiwan. Three immunoassays were selected for the evaluation based on the sentinel study results: the Roche, Abbott, and GenScript cPass™ assays. We found that the heterologous regimen ChAdOx1-S/mRNA-1273 regimen was safe, with no reported serious adverse effects, and elicited a robust antibody response.

The first proposed heterologous regimen, with the prime-boost combination of ChAdOx1-S/BNT vaccine, was assessed by Borobia et al. in a randomized controlled trial with 676 participants [12]. Several studies have been conducted in different countries to investigate the safety and immunological profile of heterologous vaccines against COVID-19 since then [17,18]. The combination of ChAdOx1-S/mRNA-1273, while less studied than ChAdOx1-S/BNT, has also demonstrated good immunogenicity in a Denmark-based observational study and a Swedish study [19,20]. Safety profile of the ChAdOx1-S/mRNA-1273 regimen was evaluated in a German study of 96 healthy participants. The reported rates of adverse events after ChAdOx1-S/mRNA-1273 was comparable to that after mRNA-1273/mRNA-1273, and was higher than that after ChAdOx1-S/ChAdOx1-S [21]. Our study also shows similar findings in that all reported adverse effects were mild or moderate [16, 22]. Although being lack of protective efficacy of effectiveness data, this "miss-match" vaccination strategy has been accepted or officially recommended by a growing number of countries, including Germany, Canada, and Thailand [23].

A comprehensive evaluation of vaccine-induced immune response can be complicated. Both B cell and T cell responses are important for vaccine-induced protection [12, 22, 24–26]. Previous studies have shown an association between measured anti-RBD/S1 antibody titers and clinical protective effects against SARS-CoV-2 [27]. However, the measured antibody levels may differ depending on the methods and target antigen used in different immunoassays [28–30]. In our study, we used three different assays to measure the levels of anti-RBD/S1 antibodies and neutralizing ability following vaccination, which is one of the strengths of this study. One of our findings is that the V4 samples of the

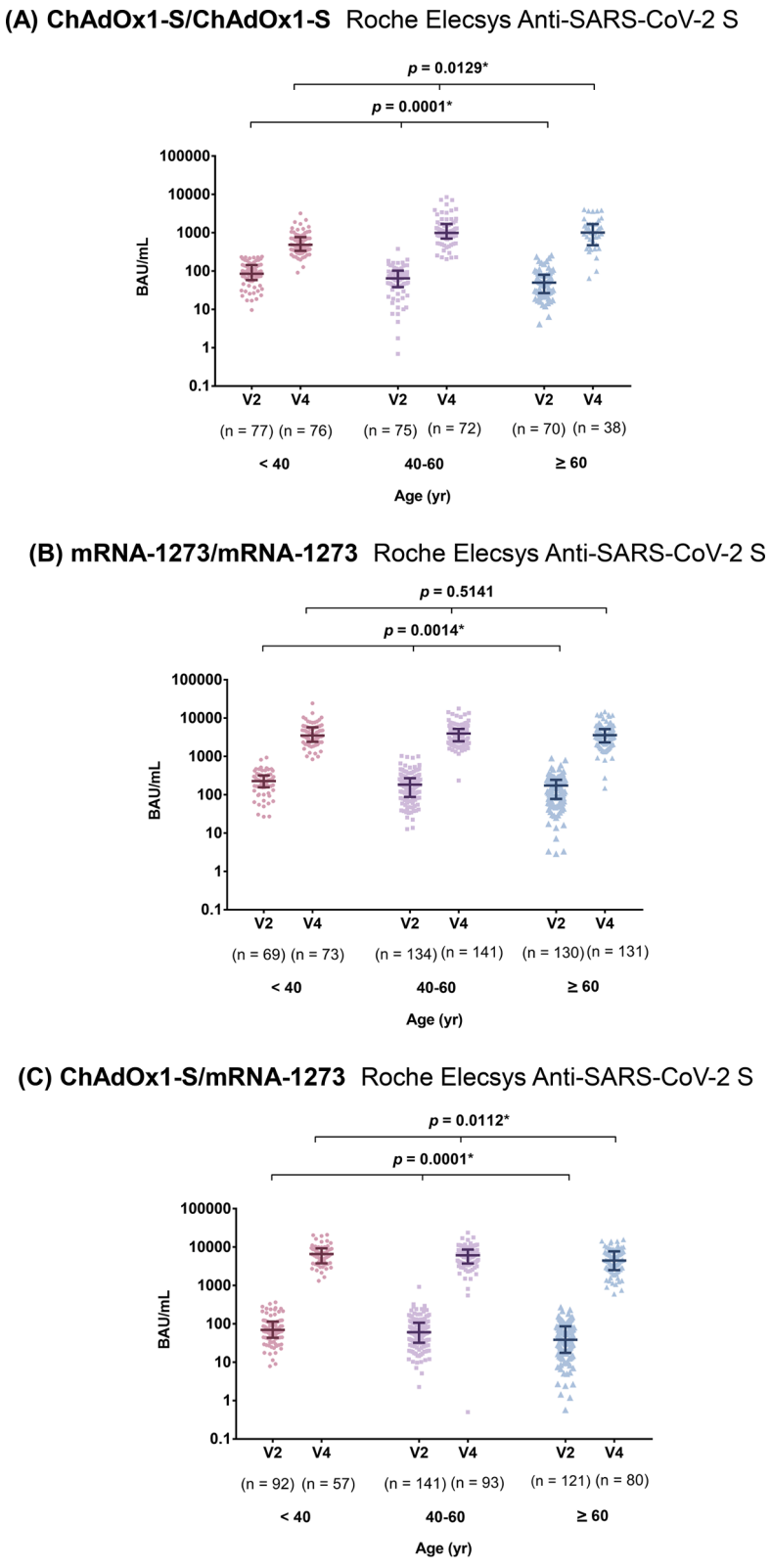

Fig. 7. Age-dependent anti-RBD/S1 antibody titers of different vaccination groups, measured by three different immunoassays (Roche Elecsys® Anti-SARS-CoV-2 S, Abbott AdviseDX SARS-CoV-2 IgG II, GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit). The whiskers denote the median (long) and the first and third interquadrant (short) values of all measurements. The analysis was done using Kruskal–Wallis test. Fig. 5(A) to Fig. 5(F) were plotted using logarithmic vertical axes. (A)(B)(C) Antibody titers of different age groups with ChAdOx1-S/ChAdOx1-S regimen, measured by Roche, Abbot, and GenScript assays, respectively. Antibody titers of different age groups with mRNA-1273/mRNA-1273 regimen, measured by Roche (D), Abbot (E), and GenScript (F) assay, respectively. Antibody titers of different age groups with ChAdOx1-S/mRNA-1273 regimen, measured by Roche (G), Abbot (H), and GenScript (I) assays, respectively.

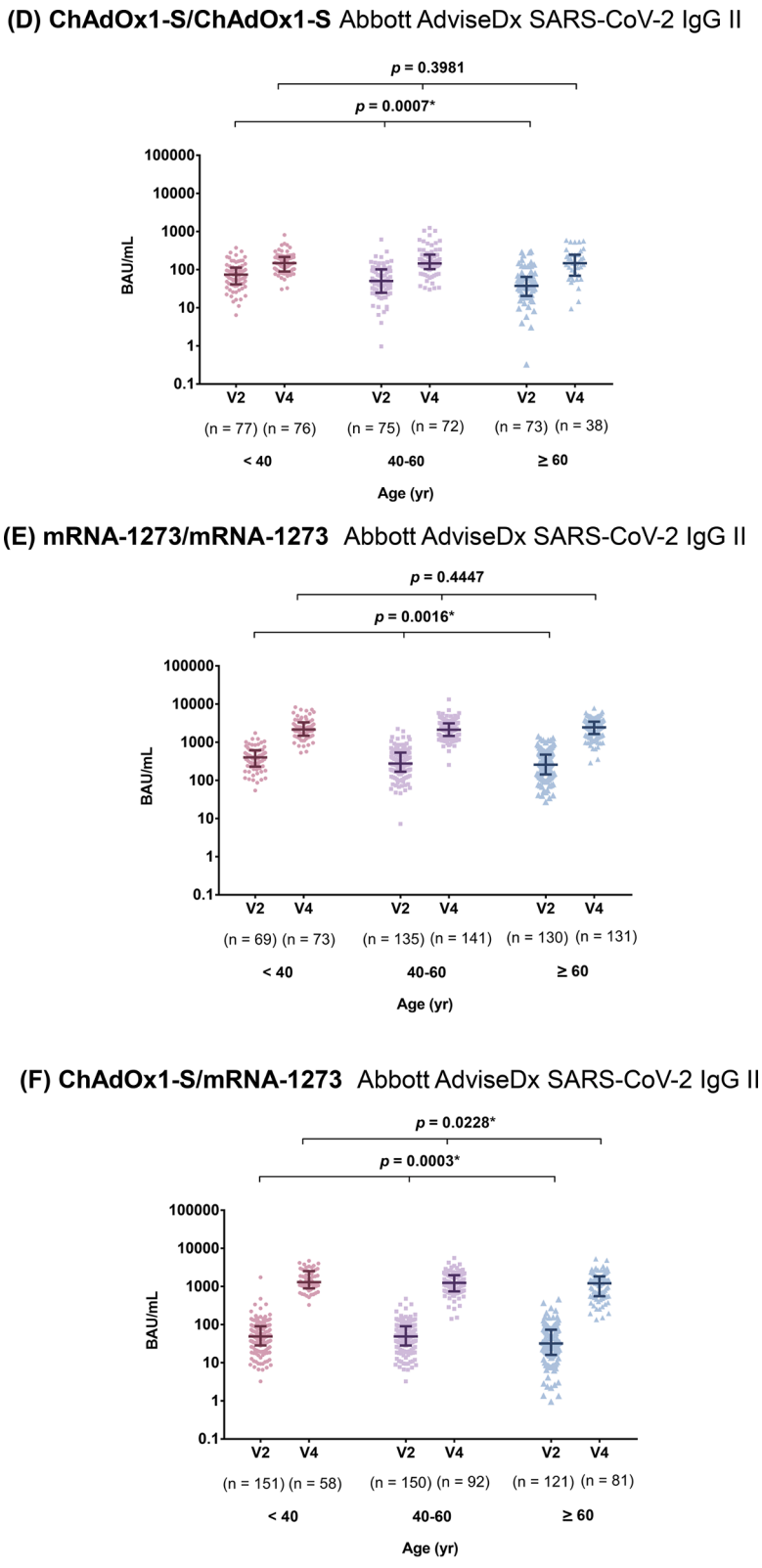

Fig. 7. (*continued*).

ChAdOx1-S/mRNA-1273 group yielded significantly higher levels of anti-RBD/S1 antibodies than homologous regimens using the Roche assay, while in the Abbott assay, the mRNA-1273/mRNA-1273 group showed higher IgG titers than the others. Considering that antigen-specific IgM usually plays a minor role, it is reasonable to infer that the difference is caused mainly by the presence of IgA, which can be detected by the Roche assay but not Abbott [31]. In previous studies, anti-SARS-CoV-2 S IgA antibodies were found in patients with COVID-19 as well as vaccinated individuals, and these antibodies also play an important role in mucosal defense against the disease [32,33]. It is likely that adenovirus-vectored vaccines have a comparable or even superior inducibility of anti-RBD/S1 IgA antibodies compared to mRNA vaccines

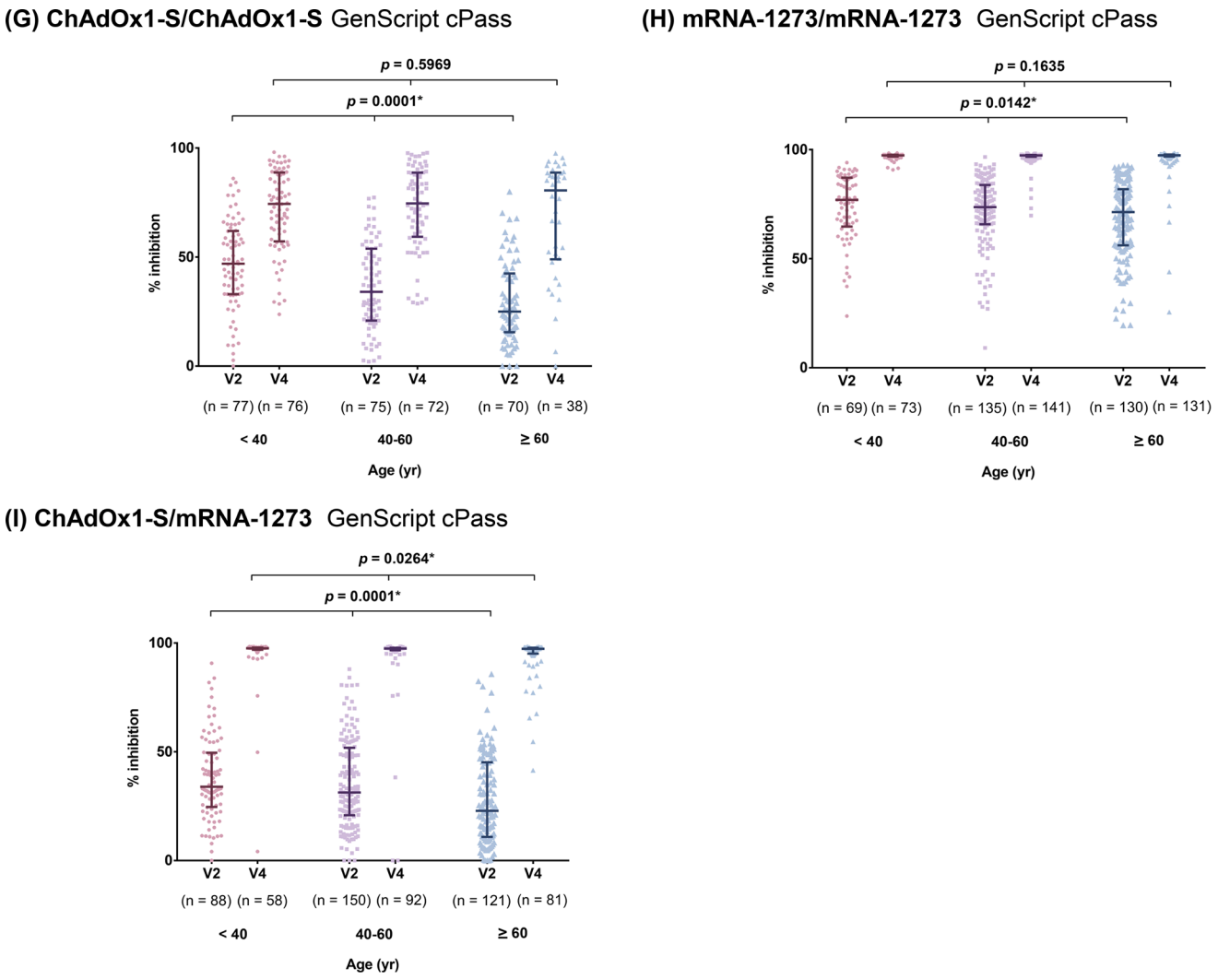

Fig. 7. (*continued*).

or vaccines from traditional platforms [12,17,18,34–36]. Further research on the humoral responses to vaccines from different platforms may provide more information for optimizing vaccination strategy.

Another finding of our study is that the immunological response to the first dose is inversely age-dependent for both ChAdOx1-S and mRNA-1273, with higher antibody titers and better neutralization abilities among younger people. After the booster dose, however, the difference was less significant among different ages, except for the ChAdOx1-S/mRNA-1273 group (Fig. 6). This result suggests a crucial role of a booster dose, especially for elderly people, to achieve a robust immunity against SARS-CoV-2 [37–39]. Interestingly, in the ChAdOx1-S/ChAdOx1-S group, the antibody titers after the booster dose (V4) were higher in older participants when measured by the Roche assay, but not by the Abbott assay (Fig. 6). A possible explanation is that more anti-vector antibodies were produced in the younger age group after the prime dose [40]. The hypothesis that anti-vector immunity after a prime dose of vectored vaccine possibly hinders the efficacy of the subsequent booster dose(s) was implied by the result from earlier trials of ChAdOx1-S in which different dosage and intervals were tested [41–43]. As repeated vaccination has been advocated for a better protection against COVID-19, the role of anti-vector immunity is an important issue for vaccine development in the future.

One limitation of this study is that humoral immunity against variant strains has not been tested. The vaccines and recombinant antigens used in the immunoassays were developed based on the original strain. The effect of vaccine-induced antibodies toward variants is questionable, especially for variants of concern that have extensive mutation sites, such as the Delta and Omicron variant [44]. A live virus or pseudovirus neutralizing assay is required to obtain such information. The neutralizing assay $TCID_{50}$ was performed in some of our samples using the Alpha variant strain (B 1.1.7/GRY clade, UK variant) as the target. Despite previous studies showed that antibodies induced by the Wuhan virus or vaccine containing Wuhan strain still retain neutralizing ability against the B 1.1.7 variant, the results in our study somehow showed otherwise [44–47]. Samples tested with $TCID_{50}$ demonstrated inadequate neutralizing ability against the B 1.1.7 variant, while the other assays suggested robust neutralizing ability and antibody titers against the original Wuhan variant. These discrepant results require further investigation. However, this indicates that no single test could be representative enough for the evaluation of humoral immunity. A combination of multiple assays may be important to provide unbiased information.

This study is the first one in Taiwan to evaluate the serological response to different regimens of SARS-CoV-2 vaccination. We found that heterologous vaccination with ChAdOx1-S/mRNA-1273 is generally safe, well-tolerated, and induces an antibody response that is non-inferior to that of mRNA-1273/mRNA-1273. Age-dependent response was seen after the prime dose, but the differences were less significant following a booster dose for both the homologous and heterologous regimens. This result suggests that boosting is crucial for a better

protection, especially in elderly people.

Funding

This work was supported by the Taiwan Centers for Disease Control and Prevention, Minister of Health and Welfare,Executive Yuan, Taiwan (YH110001).

Declaration of Competing Interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105156.

References

[1] World health organization. Situation reports: weekly epidemiological update. Available at: https://www.who.int/publications/m/item/weekly-operational-update-on-COVID -19—23-november-2021. Accessed November 25, 2021.
[2] P.I. Lee, P.R. Hsueh, Emerging threats from zoonotic coronaviruses-from SARS and MERS to 2019-nCoV, J. Microbiol. Immunol. Infect. 53 (3) (2020) 365–367.
[3] R. Jing, T. Kudinha, M.L. Zhou, M. Xiao, H. Wang, W.H. Yang, et al., Laboratory diagnosis of COVID-19 in china: a review of challenging cases and analysis, J. Microbiol. Immunol. Infect. 54 (1) (2021) 17–26.
[4] C.C. Lai, Y.H. Liu, C.Y. Wang, Y.H. Wang, S.C. Hsueh, M.Y. Yen, et al., Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): facts and myths, J. Microbiol. Immunol. Infect. 53 (3) (2020) 404–412.
[5] World health organization - vaccines/COVID-19 vaccine eul issued. Available at: https://extranet.who.int/pqweb/vaccines/vaccinesCOVID-19-vaccine-eul-issued. Accessed November 25, 2021.
[6] F.J. Tsai, H.W. Yang, C.P. Lin, J.Z. Liu, Acceptability of COVID-19 vaccines and protective behavior among adults in Taiwan: associations between risk perception and willingness to vaccinate against COVID-19, Int. J. Environ. Res. Public Health 18 (11) (2021).
[7] S.Y. Lo, S.S. Li, T.Y. Wu, Exploring psychological factors for COVID-19 vaccination intention in Taiwan, Vaccines (Basel) 9 (7) (2021).
[8] M.Y. Yen, J. Schwartz, S.Y. Chen, C.C. King, G.Y. Yang, P.R. Hsueh, Interrupting COVID-19 transmission by implementing enhanced traffic control bundling: implications for global prevention and control efforts, J. Microbiol. Immunol. Infect. 53 (3) (2020) 377–380.
[9] A. Greinacher, T. Thiele, T.E. Warkentin, K. Weisser, P.A. Kyrle, S. Eichinger, Thrombotic thrombocytopenia after ChAdOx1 nCoV-19 vaccination, N. Engl. J. Med. 384 (22) (2021) 2092–2101.
[10] A.K. Verma, K.J. Lavine, C.Y. Lin, Myocarditis after COVID-19 mRNA vaccination, N. Engl. J. Med. 385 (14) (2021) 1332–1334.
[11] E. Mathieu, H. Ritchie, E. Ortiz-Ospina, M. Roser, J. Hasell, C. Appel, et al., A global database of COVID-19 vaccinations, Nat. Hum. Behav. 5 (7) (2021) 947–953.
[12] A.M. Borobia, A.J. Carcas, M. Pérez-Olmeda, L. Castaño, M.J. Bertran, J. García-Pérez, et al., Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (combivacs): a multicentre, open-label, randomised, controlled, phase 2 trial, Lancet 398 (10295) (2021) 121–130.
[13] M. Voysey, S.A.C. Clemens, S.A. Madhi, L.Y. Weckx, P.M. Folegatti, P.K. Aley, et al., Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (azd1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK, Lancet 397 (10269) (2021) 99–111.
[14] L.R. Baden, H.M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, et al., Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine, N. Engl. J. Med. 384 (5) (2021) 403–416.
[15] S.Y. Chen, Y.L. Lee, Y.C. Lin, N.Y. Lee, C.H. Liao, Y.P. Hung, et al., Multicenter evaluation of two chemiluminescence and three lateral flow immunoassays for the diagnosis of COVID-19 and assessment of antibody dynamic responses to SARS-CoV-2 in Taiwan, Emerg. Microbes Infect. 9 (1) (2020) 2157–2168.
[16] W.P. Tseng, J.L. Wu, C.C. Wu, K.T. Kuo, C.H. Lin, M.Y. Chung, et al., Seroprevalence surveys for anti-SARS-CoV-2 antibody in different populations in Taiwan with low incidence of COVID-19 in 2020 and severe outbreaks of SARS in 2003, Front. Immunol. 12 (2021), 606629.
[17] T.C. Ho, Y.A. Chen, H.P. Chan, C.C. Chang, K.P. Chuang, C.H. Lee, et al., The effects of heterologous immunization with prime-boost COVID-19 vaccination against SARS-CoV-2, Vaccines (Basel) 9 (10) (2021).
[18] D. Fabricius, C. Ludwig, J. Scholz, I. Rode, C. Tsamadou, E.M. Jacobsen, et al., mRNA vaccines enhance neutralizing immunity against SARS-CoV-2 variants in convalescent and ChAdOx1-primed subjects, Vaccines (Basel) 9 (8) (2021).
[19] M.A. Gram, J. Nielsen, A.B. Schelde, K.F. Nielsen, I.R. Moustsen-Helms, A. K. Bjørkholt Sørensen, et al., Vaccine effectiveness when combining the ChAdOx1 vaccine as the first dose with an mRNA COVID-19 vaccine as the second dose, medRxiv (2021), https://doi.org/10.1101/2021.07.26.21261130.
[20] P. Nordstrom, M. Ballin, A. Nordstrom, Effectiveness of heterologous ChAdOx1 nCoV-19 and mRNA prime-boost vaccination against symptomatic COVID-19 infection in Sweden: a nationwide cohort study, Lancet Reg. Health Eur. 11 (2021), 100249.
[21] T. Schmidt, V. Klemis, D. Schub, J. Mihm, F. Hielscher, S. Marx, et al., Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination, Nat. Med. 27 (9) (2021) 1530–1535.
[22] C.J. Wang, C.Y. Ng, R.H. Brook, Response to COVID-19 in Taiwan: big data analytics, new technology, and proactive testing, JAMA 323 (14) (2020) 1341–1342.
[23] E. Callaway, Mix-and-match COVID vaccines trigger potent immune response, Nature 593 (7860) (2021) 491.
[24] A. Hjelholt, G. Christiansen, U.S. Sorensen, S. Birkelund, IgG subclass profiles in normal human sera of antibodies specific to five kinds of microbial antigens, Pathog. Dis. 67 (3) (2013) 206–213.
[25] K.J. Ewer, J.R. Barrett, S. Belij-Rammerstorfer, H. Sharpe, R. Makinson, R. Morter, et al., T cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (azd1222) vaccine in a phase 1/2 clinical trial, Nat. Med. 27 (2) (2021) 270–278.
[26] L.A. Jackson, E.J. Anderson, N.G. Rouphael, P.C. Roberts, M. Makhene, R.N. Coler, et al., An mRNA vaccine against SARS-CoV-2 - preliminary report, N. Engl. J. Med. 383 (20) (2020) 1920–1931.
[27] S. Feng, D.J. Phillips, T. White, H. Sayal, P.K. Aley, S. Bibi, et al., Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection, bioRxiv (2021), https://doi.org/10.1101/2021.06.21.21258528.
[28] S. Jeong, N. Lee, S.K. Lee, E.J. Cho, J. Hyun, M.J. Park, et al., Comparing results of five SARS-CoV-2 antibody assays before and after the first dose of ChAdOx1 nCoV-19 vaccine among health care workers, J. Clin. Microbiol. 59 (9) (2021), e0110521.
[29] K. Saker, V. Escuret, V. Pitiot, A. Massardier-Pilonchery, S. Paul, B. Mokdad, et al., Evaluation of commercial anti-SARS-CoV-2 antibody assays and comparison of standardized titers in vaccinated healthcare workers, J. Clin. Microbiol. (2021), https://doi.org/10.1128/JCM.01746-21:JCM0174621.
[30] Y.C. Lin, Y.L. Lee, C.Y. Cheng, W.P. Tseng, J.L. Wu, C.H. Lin, et al., Multicenter evaluation of four immunoassays for the performance of early diagnosis of COVID-19 and assessment of antibody responses of patients with pneumonia in Taiwan, J. Microbiol. Immunol. Infect. 54 (5) (2021) 816–829.
[31] R.B.M. Schasfoort, J. van Weperen, M. van Amsterdam, J. Parisot, J. Hendriks, M. Koerselman, et al., Presence and strength of binding of IgM, IgG and IgA antibodies against SARS-CoV-2 during COVID-19 infection, Biosens. Bioelectron. 183 (2021), 113165.
[32] A. Padoan, L. Sciacovelli, D. Basso, D. Negrini, S. Zuin, C. Cosma, et al., IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: a longitudinal study, Clin. Chim. Acta 507 (2020) 164–166.
[33] S. Zurac, L. Nichita, B. Mateescu, C. Mogodici, A. Bastian, C. Popp, et al., COVID-19 vaccination and IgG and IgA antibody dynamics in healthcare workers, Mol. Med. Rep. 24 (2) (2021).
[34] J. Normark, L. Vikstrom, Y.D. Gwon, I.L. Persson, A. Edin, T. Bjorsell, et al., Heterologous ChAdOx1 nCoV-19 and mRNA-1273 vaccination, N. Engl. J. Med. 385 (11) (2021) 1049–1051.
[35] N. Wanlapakorn, N. Suntronwong, H. Phowatthanasathian, R. Yorsaeng, P. Vichaiwattana, T. Thongmee, et al., Safety and immunogenicity of heterologous and homologous inactivated and adenoviral-vectored COVID-19 vaccines in healthy adults, medRxiv (2021), https://doi.org/10.1101/2021.11.04.21265908.
[36] E.C. Lavelle, R.W. Ward, Mucosal vaccines - fortifying the frontiers, Nat. Rev. Immunol. (2021), https://doi.org/10.1038/s41577-021-00583-2.
[37] D.A. Collier, I. Ferreira, P. Kotagiri, R.P. Datir, E.Y. Lim, E. Touizer, et al., Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2, Nature (2021), https://doi.org/10.1038/s41586-021-03739-1.
[38] K.L. Bajema, R.M. Dahl, M.M. Prill, E. Meites, M.C. Rodriguez-Barradas, V. C. Marconi, et al., Effectiveness of COVID-19 mRNA vaccines against COVID-19-associated hospitalization - five veterans affairs medical centers, United States, February 1-August 6, 2021, MMWR Morb. Mortal. Wkly. Rep. 70 (37) (2021) 1294–1299.
[39] M.A. Brockman, F. Mwimanzi, H.R. Lapointe, Y. Sang, O. Agafitei, P. Cheung, et al., Reduced magnitude and durability of humoral immune responses by COVID-19 mRNA vaccines among older adults, medRxiv (2021), https://doi.org/10.1101/2021.09.06.21263149.
[40] S.A. Mendonca, R. Lorincz, P. Boucher, D.T. Curiel, Adenoviral vector vaccine platforms in the SARS-CoV-2 pandemic, NPJ Vaccines 6 (1) (2021) 97.
[41] L. Zamai, M.B.L. Rocchi, Hypothesis: possible influence of antivector immunity and SARS-CoV-2 variants on efficacy of ChAdOx1 nCoV-19 vaccine, Br. J. Pharmacol. (2021), https://doi.org/10.1111/bph.15620.
[42] M.N. Ramasamy, A.M. Minassian, K.J. Ewer, A.L. Flaxman, P.M. Folegatti, D. R. Owens, et al., Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (Cov002): a single-blind, randomised, controlled, phase 2/3 trial, Lancet 396 (10267) (2020) 1979–1993.
[43] M. Voysey, S.A. Costa Clemens, S.A. Madhi, L.Y. Weckx, P.M. Folegatti, P.K. Aley, et al., Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (azd1222) vaccine: a pooled analysis of four randomised trials, Lancet 397 (10277) (2021) 881–891.
[44] G.M.N. Behrens, A. Cossmann, M.V. Stankov, I. Nehlmeier, A. Kempf, M. Hoffmann, et al., SARS-CoV-2 delta variant neutralisation after heterologous ChAdOx1-S/BNT162b2 vaccination, Lancet 398 (10305) (2021) 1041–1042.

[45] D. Planas, T. Bruel, L. Grzelak, F. Guivel-Benhassine, I. Staropoli, F. Porrot, et al., Sensitivity of infectious SARS-CoV-2 b.1.1.7 and b.1.351 variants to neutralizing antibodies, Nat. Med. 27 (5) (2021) 917–924.
[46] V.V. Edara, W.H. Hudson, X. Xie, R. Ahmed, M.S. Suthar, Neutralizing antibodies against SARS-CoV-2 variants after infection and vaccination, JAMA 325 (18) (2021) 1896–1898.
[47] N. Kohmer, C. Ruhl, S. Ciesek, H.F. Rabenau, Utility of different surrogate enzyme-linked immunosorbent assays (selisas) for detection of SARS-CoV-2 neutralizing antibodies, J. Clin. Med. 10 (10) (2021).