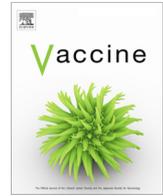




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Immunogenicity and reactogenicity after booster dose with AZD1222 via intradermal route among adult who had received CoronaVac



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ABSTRACT

Background: Currently, booster dose is needed after 2 doses of non-live COVID-19 vaccine. With limited resources and shortage of COVID-19 vaccines, intradermal (ID) administration might be a potential dose-sparing strategy.

Objective: To determine immunologic response and reactogenicity of ID ChAdOx1 nCoV-19 vaccine (AZD1222, Oxford/AstraZeneca) as a booster dose after completion of 2-dose CoronaVac (SV) in healthy adult.

Methods: This is a prospective cohort study of adult aged 18–59 years who received 2-dose SV at 14–35 days apart for more than 2 months. Participants received ID AZD1222 at fractional low dose (1×10^{10} viral particles, 0.1 ml). Antibody responses were evaluated by surrogate virus neutralization test (sVNT) against delta variant and wild type, and anti-spike-receptor-binding-domain immunoglobulin G (anti-S-RBD IgG) at prior, day 14, 28, 90, and 180 post booster. Solicited reactogenicity was collected for 7 days post-booster. Primary endpoint was the differences of sVNT against delta strain $\geq 80\%$ inhibition at day 14 and 90 compared with the parallel cohort study of 0.5-ml intramuscular (IM) route.

Results: From August 2021, 100 adults with median age of 46 years (IQR 41–52) participated. Prior to booster, geometric mean (GM) of sVNT against delta strain was 22.4% inhibition (95% CI 18.7–26.9) and of anti-S-RBD IgG was 109.3 BAU/ml (95.4–125.1). Post ID booster, GMs of sVNT against delta strain were 95.5% inhibition (95% CI 94.2–96.8) at day 14, 73.1% inhibition (66.7–80.2) at day 90, and 22.7% inhibition (14.9–34.6) at day 180. The differences of proportion of participants achieving sVNT against delta strain $\geq 80\%$ inhibition in ID recipients versus IM were +4.2% (95% CI –2.0 to 10.5) at day 14, and –37.3% (–54.2 to –20.3) at day 90. Anti-S-RBD IgG GMs were 2037.1 BAU/ml (95% CI 1770.9–2343.2) at day 14 and 744.6 BAU/ml (650.1–852.9) at day 90, respectively. Geometric mean ratios (GMRs) of

Abbreviations: BAU, Binding-antibody unit; BMI, Body mass index; CMI, Cell-mediated immunity; ELISpot, Enzyme-linked immunospot; GM, Geometric mean; GMR, Geometric mean ratio; ID, Intradermal; IM, Intramuscular; PBMC, Peripheral blood mononuclear cell; SFU, Spot forming unit; S-RBD, Spike receptor binding domain; sVNT, Surrogate virus neutralization test.

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anti-S-RBD IgG were 0.99 (0.83–1.20) at day 14, and 0.82 (0.66–1.02) at day 90. Only 18% reported feverish, compared with 37% of IM ($p = 0.003$). Common reactogenicity was erythema at injection site (53%) while 7% reported blister.

Conclusion: Low-dose ID AZD1222 booster enhanced lower neutralizing antibodies at 3 months compared with IM route. Less systemic reactogenicity occurred, but higher local reactogenicity.

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1. Introduction

Over 250 million cases of Coronavirus disease (COVID-19) were reported worldwide with more than 5 million deaths [1], despite over 7 billion doses of vaccines administered. In Thailand, as of November 2021, more than 2 million cases of COVID-19 were reported with over 20,000 deaths. Non-live COVID-19 vaccine, CoronaVac (Sinovac Life Sciences, Beijing, China), was used for mass vaccination in several countries e.g., Thailand, China, Brazil, and Chile. Effectiveness of CoronaVac for prevention of COVID-19 was 65.9% from study in Chile [2] and 36.8% from study in Brazil [3]. With the rising of delta variant (B.1.617.2) of SARS-CoV-2 globally, the neutralizing activity induced by CoronaVac declined [4]. Heterologous prime-boost vaccination may provide better immunogenicity. With AZD1222 followed by BNT162b2 heterologous prime-boost vaccination, this vaccination strategy provided highest T cell responses compared with homologous vaccination [5,6].

Standard administration of currently available COVID-19 vaccine is via intramuscular injection. Potential routes for vaccine administration could be intramuscular (IM) or intradermal (ID) administration in which efficacy is related to the immunogenicity [7]. ID administration offers potential dose-sparing benefit compared with intramuscular administration, rabies vaccination as an example. ID vaccination is a technique in which the vaccine is administered into dermis which is rich in antigen presenting cells such as dermal dendritic cells [8]. Because of the abundance of antigen presenting cells in skin, ID administration required less antigenic dose (usually 20%–30% of standard dose) to induce comparable immune responses to standard IM vaccination. Many studies showed effective immune response by ID administration of influenza, rabies, hepatitis B, Bacille Calmette-Guerin (BCG), and polio vaccines [7,9–12]. For influenza vaccine, a systematic review and meta-analysis showed comparable seroprotection rates for 9- μ g ID with 15- μ g IM injection with higher local adverse events particularly erythema and swelling [9]. For rabies vaccine, ID schedules offered advantages through saving in costs, doses, and time as recommended by WHO, and were approved use on label of vaccine [12].

Fractionated-dose ID COVID-19 vaccine is potential for rapid achievement of herd immunity based on other vaccines reported [7]. Study of one-tenth dose of mRNA-1273 ID vaccination showed comparable anti-spike IgG and anti-receptor-binding-domain (anti-RBD) IgG responses to conventional IM vaccination at 2 weeks post primary vaccination series [13]. However, one-fifth dose of BNT162b2 ID booster in healthy Thai adult post 2-dose CoronaVac failed to boost T cell response at 14 days, despite robust neutralizing antibodies response [14]. A case report of ID AZD1222 after 2 doses of CoronaVac showed increase of antibodies, T cell responses against spike protein, and neutralizing antibody to almost 100% at 2–3 weeks after booster with minimal local reaction [15].

This study aims to evaluate immunogenicity and reactogenicity of ID AZD1222 booster dose in adults who had received 2 doses of CoronaVac.

2. Methods

2.1. Study design and participants

This study was conducted at Chulalongkorn University Health Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. This is a prospective cohort study. The participants who aged 18 – 59 years old and received two doses of CoronaVac for at least 60 days, 14 – 35 days apart were included in this study. The exclusion criteria were receiving any immunosuppressants or blood products within 3 months before the enrollment or receiving any vaccines within 2 weeks. All participants gave written informed consent prior to study enrollment.

This study was registered in Thai Clinical Trials Registry (thaiclinicaltrials.org, TCTR 20210817003). Immunogenicity parameters were compared with the parallel randomized controlled trial on healthy adult with standard dose and low dose IM administration of AZD1222 booster after completing 2 doses of CoronaVac (TCTR20210722003), conducted at same settings and lab. We compared the results of this study with conventional standard dose (0.5 ml) IM group at the same study site (Chulalongkorn University). Institutional review board of Faculty of Medicine, Chulalongkorn University approved this study (IRB no. 663/64) and parallel IM AZD1222 booster study (IRB no. 600/64).

2.2. Study procedures

One hundred participants were recruited in this study. At baseline, the history of SARS-CoV-2 vaccination and exposure to confirmed COVID-19 case within 3 months was taken. Blood sample was collected prior to giving a booster dose. The participants received ID AZD1222 lot number A1009, manufactured by Siam Bioscience Co., Ltd., 0.1 ml (1×10^{10} viral particles). The ID vaccination was performed by trained physician/nurse (RN and TT) at the deltoid area using Mantoux technique [16]. The solicited local and systemic reactogenicity during 7 days after vaccination was recorded in the diary. The solicited reactogenicity included pain, swelling, erythema, fever, headache, malaise, myalgia, arthralgia, vomiting, and diarrhea. Scheduled visits, as shown in Fig. 1, were day 14 for 50 participants, day 28 for 50 participants, day 90 for 40 participants, and day 180 for 60 participants, to collect reactogenicity data and perform blood collection.

The cell-mediated immunity (CMI) sub study was performed among 20 participants, selected by the order of enrollment, with enzyme-linked immunospot (ELISpot) assay at baseline, day 28, day 90, and day 180 to evaluate T and B cell responses.

2.3. Immunogenicity outcomes

All participants' samples were tested for anti-spike-receptor-binding-domain (anti-S-RBD) IgG, and functional neutralizing antibody (NAbs) against SARS-CoV-2 wild type and delta variants by surrogate virus neutralization test (sVNT). All of the immunogenic-

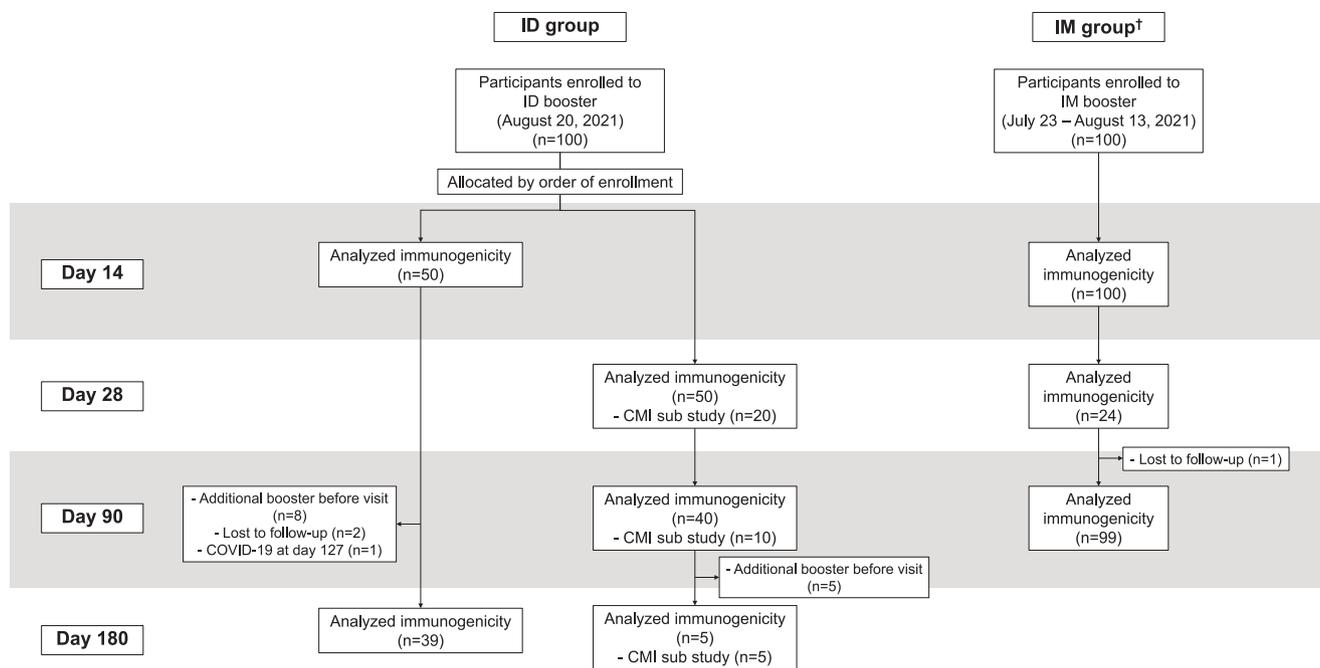


Fig. 1. Flow diagram of study participants. CMI: Cell-mediated immunity, ID: Intradermal, IM: Intramuscular, [†]IM group from standard-dose AZD1222 booster in healthy adult completing 2-dose CoronaVac at the same study site (Chulalongkorn University, Bangkok, Thailand) of Nanthapaisal S, et al. A randomized clinical trial of a booster dose with low versus standard dose of AZD1222 in adult after 2 doses of inactivated vaccines. Vaccine 2022. <https://doi.org/10.1016/j.vaccine.2022.03.036>.

ity results in ID group were compared with IM participants at equivalent time points.

2.3.1. Quantitative spike receptor binding domain IgG (anti-S-RBD IgG) ELISA

The ELISA protocol was adapted from Amanat *et al.* (2020) [17]. Briefly, diluted serum samples were incubated in 96-well plates coated with purified recombinant Myc-His-tagged S-RBD, residues 319–541 from SARS-CoV-2 (Wuhan-Hu-1). Then, ELISA was performed. Anti-S-RBD IgG level was reported in binding-antibody units (BAU/mL) following conversion of OD450 values with the standard curve using known units of WHO international standard (NIBSC 20/136). We used anti-S-RBD IgG level at 506 BAU/ml, which is correlated with 80% vaccine efficacy reported by the Oxford COVID vaccine trial group [18], as a cut off.

2.3.2. Surrogate virus neutralization test (sVNT)

A surrogate virus neutralization test was set up as previously described in Tan *et al.* (2020) [19]. Recombinant SRBD from the wild-type (Wuhan-Hu-1) and delta (B.1.617.2) strains were used. Serum samples - SRBD mixture were incubated in 96-well plates coated with 0.1 µg/well recombinant human ACE2 ectodomain (GenScript). Then, ELISA was performed. The negative sample was pre-2019 human serum. The % inhibition was calculated as follows:

$$\%inhibition = 100 \times \left[1 - \frac{sampleOD450}{negativeOD450} \right]$$

2.3.3. Enzyme-linked immunospot (ELISpot) assay to evaluate T and B cell responses

For T cell, ELISpot assay using a Human IFN-γ ELISpotPro™ kit (Mabtech, Stockholm, Sweden) was used for SARS-CoV-2-specific T cell responses in fresh peripheral blood mononuclear cells (PBMCs). Briefly, 2.5 × 10⁵ PBMCs were stimulated in AIM-V medium with overlapping peptide pool from 100 peptides of SARS-

CoV-2 Spike (S) defined peptides and 101 peptides from the nucleoprotein (N), membrane protein (M), open reading frame proteins (O) (Mabtech, Stockholm, Sweden) at a final concentration of 2 µg/ml for 20 hours. Negative control and positive control, anti-CD3, were also included. The spots were counted using ImmunoSpot analyzer. Spot counts for negative control wells were subtracted from the test wells to generate normalized readings, these are presented as spot forming unit (SFU) per million PBMCs.

For B cells, Human IgG SARS-CoV-2 RBD ELISpot PLUS (ALP) kit (Mabtech, Stockholm, Sweden) was used for SARS-CoV-2-specific B cell responses. Briefly, the memory B cells were differentiated into antibody secreting cells by pre-stimulating the fresh PBMCs with R848 and IL-2 for 72 hours. Unstimulated well was also used as negative control. Stimulated and unstimulated PBMCs (5 × 10⁵ cells per well) were added into ELISpot plate and incubated for 18 hours. An RBD-WASP antigen was added into RBD-specific IgG detected well while MT78/145- biotinylated antibodies were added into total IgG detected well, positive control. Anti-WASP-ALP was added into RBD-specific IgG detected well and negative control well while streptavidin-ALP was added into total IgG detected well. Spot counting was performed in the same method as T cells.

2.4. Reactogenicity

Solicited reactogenicity was recorded by participants using diary. All symptoms were graded in 3 grades [20]: grade 0 for no symptom; grade 1 for mild symptom, which was not interfere with activities or vomiting 1 – 2 times/day or diarrhea 2 – 3 times/day; grade 2 for moderate symptom, which interfered with activities or need to take medication, or vomiting more than 2 times/day or diarrhea 4 – 5 times/day; grade 3 for severe symptom, which incapacitated or need hospitalization or diarrhea 6 or more times/day. Fever was graded as grade 1 (38.0 – 38.4 °C), grade 2 (38.5 – 38.9 °C), grade 3 (39 – 40 °C), and grade 4 (more than 40 °C). Unsolicited adverse events were also recorded at all visits by study team.

Table 1
Baseline characteristics of participants receiving AZD1222 booster vaccine after 2-dose CoronaVac in healthy adults.

Characteristics	ID (N = 100)	IM [†] (N = 100)	p-value
Gender			
Female, n (%)	45 (45)	61 (61)	0.02 [‡]
Age (years)	46 (41–52)	45 (34–50)	0.08 [‡]
BMI (kg/m ²)	24.8 (21.4–27.4)	24.1 (21.6–26.9)	0.37 [‡]
Underlying disease, n (%)	19 (19)	29 (29)	0.10 [‡]
Hypertension, n (%)	7 (7)	9 (9)	0.60 [‡]
Dyslipidemia, n (%)	6 (6)	7 (7)	0.77 [‡]
Diabetes mellitus, n (%)	6 (6)	3 (3)	0.31 [‡]
Allergic rhinitis, n (%)	1 (1)	2 (2)	0.56 [‡]
Duration of 2 nd doses of CoronaVac and AZD1222 (days)	71 (65–76)	66 (62–74)	0.002 ^{‡*}
Immunogenicity at baseline prior to booster			
• sVNT-delta (% inhibition), GM (95% CI)	22.4 (18.7–26.9)	17.9 (14.3–22.5)	0.14 [§]
• sVNT-WT (% inhibition), GM (95% CI)	44.5 (40.6–48.7)	41.8 (37.1–47.1)	0.41 [§]
• Anti-S-RBD (BAU/ml), GM (95% CI)	109.3 (95.4–125.1)	98.9 (85.8–113.9)	0.31 [§]
• SNMO-specific T cell response (SFU/10 ⁶ PBMCs)	32 (14–56)	52 (40–84)	0.05 [‡]
	(n = 20)	(n = 19)	
• RBD-specific B cell response (SFU/10 ⁶ PBMCs)	2 (0–10)	4 (0–16)	0.50 [‡]
	(n = 20)	(n = 19)	

All data are reported as median (IQR), unless otherwise indicated.

Anti-S-RBD: Anti-spike-receptor-binding-domain, BAU: Binding-antibody unit, BMI: Body mass index, GM: Geometric mean, ID: Intradermal, IM: Intramuscular, IQR: Interquartile range, RBD: Receptor binding domain, SNMO: Spike (S) nucleoprotein (N), membrane protein (M), and open reading frame proteins (O) of SARS-CoV-2, sVNT-delta: Surrogate virus neutralization test against delta strain, sVNT-WT: Surrogate virus neutralization test against wild type.

[‡] Chi-square.

[‡] Wilcoxon rank sum test.

[§] Two sample independent t test *p < 0.05.

[†] IM group from standard-dose AZD1222 booster in healthy adult completing 2-dose CoronaVac at the same study site (Chulalongkorn University, Bangkok, Thailand) of Nanthapaisal S, et al. A randomized clinical trial of a booster dose with low versus standard dose of AZD1222 in adult after 2 doses of inactivated vaccines. Vaccine 2022. <https://doi.org/10.1016/j.vaccine.2022.03.036>.

2.5. Statistical analysis

Demographic and clinical characteristics were described for the subjects. Continuous variables were expressed as median (interquartile range: IQR) and number with percentage for categorical variables. Differences in continuous and categorical variables between two groups were assessed using a Wilcoxon rank sum test, Chi-square test, or Fisher exact test, respectively. The sVNT results, against either wild type or delta strain, of more than 80% were used to classify the achievement of 80% protection against symptomatic infection. The anti-S-RBD IgG of more than 506 BAU/ml was used in this study as a cut-off for protective antibody level, which previously reported to be associated with 80% vaccine efficacy against primary symptomatic COVID-19 [18].

We presented primary comparisons between ID and IM group in terms of the differences of proportion of participants achieving sVNT ≥ 80% inhibition. Non-inferiority was concluded if the lower bound of the 95% confidence interval (95% CI) did not exceed –10%. Geometric means (GMs) and geometric mean ratios (GMRs) with 95% CI of sVNT and anti-S-RBD IgG at day 0, 14, 28, and 90 after booster vaccination, were calculated. Non-inferiority was concluded if the lower bound of the 95% CI did not exceed 0.67. All P-values reported are two-sided. Statistical significance was defined as P < 0.05. Stata version 15.1 (Stata Corp., College Station, Texas) was used for analysis.

By using a non-inferiority criterion, the sample size was calculated from the GMR of sVNT against wild type and delta variant, and anti-S-RBD IgG, comparing ID with IM group. Assuming GMR of 0.95 with standard deviation of 1.4, 0.67 non-inferiority margin, 90% power, and one-sided statistical testing with 5% significance level, and ratio of 1:2, a minimum of 44 participants in ID and 88 for IM were required. Considering potentially missing data, the sample size was increased by 10%, yielding 50 participants for ID and 100 for IM.

3. Results

3.1. Baseline characteristics

The participants were enrolled during August 2021. The demographic data was shown in Table 1. The median age was 46 years (IQR 41–52), and 55% were male. Underlying disease was described, as shown in Table 1. Duration between 2 doses of CoronaVac was 21 days with median of 71 days (IQR 65–76) prior to ID booster administration. The GM of sVNT against delta strain was 22.4% inhibition and against wild type was 44.5% inhibition. The number of females were higher in IM cohort, while the interval between completion of 2-dose CoronaVac and AZD1222 booster was longer in ID than IM cohort. The flow diagram of participants was shown in Fig. 1.

3.2. Reactogenicity after ID AZD1222 booster

The most common solicited reactogenicity was localized at injection site such as erythema and pain, as shown in Fig. 2 and Supplementary Table 1. More than half (53%) of participants reported erythema, lasting for median duration of 4 days (IQR 3–6), which was mostly grade 1. Pain at injection site was reported in 43% with median duration of 2 days (IQR 1–4) and also mostly grade 1. Other solicited reactogenicity reported were fatigue (40%), myalgia (30%), headache (27%), feverish (18%), swelling (17%), arthralgia (12%) and diarrhea (9%). Vesicle and blister at injection site, which progressed to dry blister and turned to hyperpigmentation, were also reported as unsolicited reactogenicity (photo as shown in Supplementary Fig. 1).

Lower systemic reactogenicity including fever (0% ID versus 27% IM, p = N/A), feverish (18% ID versus 37% IM, p = 0.003), headache (27% ID versus 64% IM, p < 0.001), fatigue (40% ID versus 68% IM,

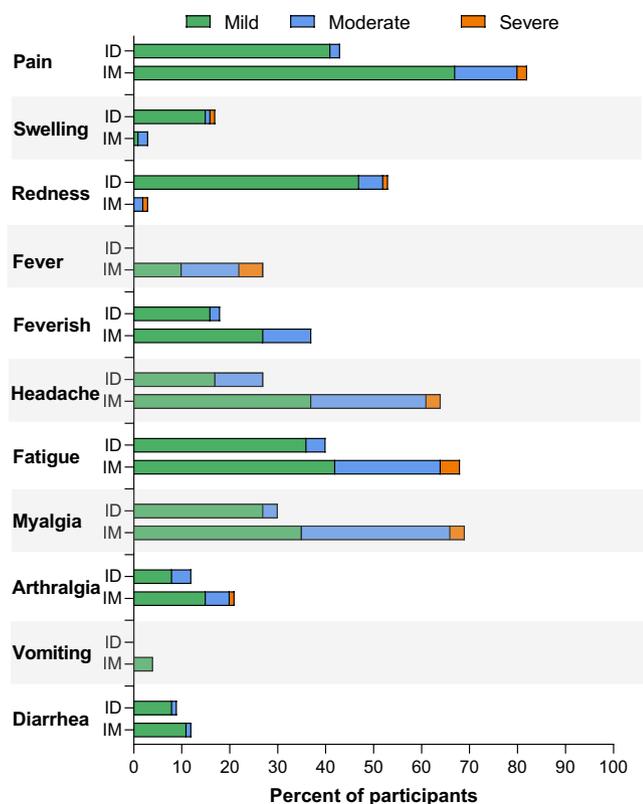


Fig. 2. Solicited reactogenicity within 7 days of ID and IM[†] AZD1222 booster after 2-dose CoronaVac in healthy adult. ID: Intradermal, IM: Intramuscular, [†]IM group from standard-dose AZD1222 booster in healthy adult completing 2-dose CoronaVac at the same study site (Chulalongkorn University, Bangkok, Thailand) of Nanthapaisal S, et al. A randomized clinical trial of a booster dose with low versus standard dose of AZD1222 in adult after 2 doses of inactivated vaccines. Vaccine 2022. <https://doi.org/10.1016/j.vaccine.2022.03.036>.

$p < 0.001$), and myalgia (30% ID versus 69% IM, $p < 0.001$) was reported in ID compared with IM, as shown in [Supplementary Table 1](#).

3.3. Immunogenicity after ID AZD1222 booster

3.3.1. sVNT against delta strain and wild type after ID AZD1222 booster

The GMs (95% CI) of sVNT against delta strain were 95.5% inhibition (94.2–96.8) at day 14, and 93.7% inhibition (91.9–95.5) at day 28 after ID booster, as shown in [Fig. 3](#) and [Table 2](#). The GMs (95% CI) of sVNT against wild type were 94.8% inhibition (94.0–95.6) at day 14, and 93.7% inhibition (92.1–95.4) at day 28, as shown in [Table 2](#). The GMs of sVNT against delta strain and wild type waned to 73.1% inhibition (95% CI 66.7–80.2) and 81.9% inhibition (76.2–88.0) after 90 days, and further waned to 22.7% inhibition (14.9–34.6) and 40.8% inhibition (31.2–53.4) after 180 days, respectively.

Ninety-eight percent of the participants achieved sVNT against delta strain and wild type $\geq 80\%$ inhibition at both day 14 and day 28. After 90 days, the proportion of participants dropped to 52.6% and 68.4%, respectively. At 180 days post ID booster, only 11.4% and 20.4% of participants had sVNT against delta strain and wild type $\geq 80\%$ inhibition.

3.3.2. Anti-S-RBD IgG after ID AZD1222 booster

The GMs (95% CI) of anti-S-RBD IgG were 2037.1 (1770.9–2343.2), 1084.9 (970.0–1213.4), 744.6 (650.1–852.9), and 330.7 (266.3–410.8) BAU/mL at day 14, 28, 90, and 180 after ID booster, respectively, as shown in [Table 2](#).

3.4. Immunogenicity of ID AZD1222 booster compared with IM

Proportions of participants with sVNT against delta strain $\geq 80\%$ inhibition at day 14 and 28 were non-inferior among ID recipients

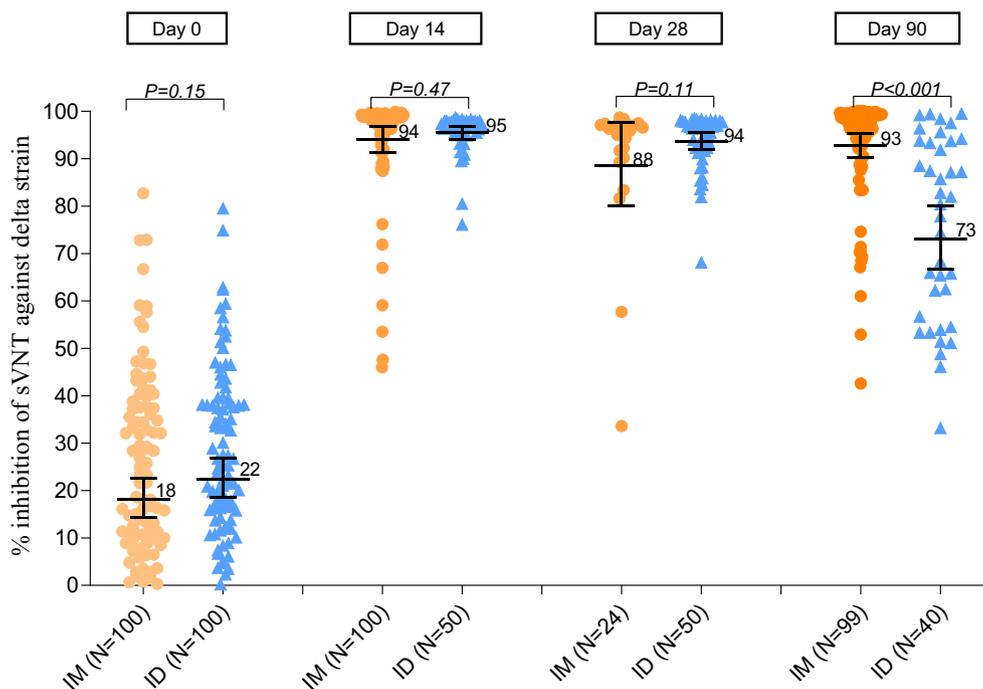


Fig. 3. Geometric means (95% CI) of sVNT against delta strain (% inhibition) at day 0, 14, 28, and 90 of ID and IM[†] AZD1222 booster after 2-dose CoronaVac in healthy adult. P-value was evaluated by two sample independent *t* test. ID: Intradermal, IM: Intramuscular, sVNT: Surrogate virus neutralization test [†]IM group from standard-dose AZD1222 booster in healthy adult completing 2-dose CoronaVac at the same study site (Chulalongkorn University, Bangkok, Thailand) of Nanthapaisal S, et al. A randomized clinical trial of a booster dose with low versus standard dose of AZD1222 in adult after 2 doses of inactivated vaccines. Vaccine 2022. <https://doi.org/10.1016/j.vaccine.2022.03.036>.

Table 2
Comparison of intradermal and intramuscular AZD1222 booster immunogenicity in healthy adult completing 2-dose CoronaVac.

	ID GM (95% CI)	IM [†] GM (95% CI)	GMR (95% CI)
Day 14	N = 50	N = 100	
· sVNT-delta (% inhibition)	95.5 (94.2–96.8)	94.7 (92.4–97.1)	1.01 (0.97–1.04)
· sVNT-WT (% inhibition)	94.8 (94.0–95.6)	96.7 (94.9–98.4)	0.98 (0.96–1.01)
· Anti-S-RBD (BAU/ml)	2037.1 (1770.9–2343.2)	2043.2 (1824.5–2288.2)	0.99 (0.83–1.20)
Day 28	N = 50	N = 24	
· sVNT-delta (% inhibition)	93.7 (91.9–95.5)	88.5 (80.1–97.7)	1.06 (0.99–1.14)
· sVNT-WT (% inhibition)	93.7 (92.1–95.4)	91.5 (85.9–97.4)	1.02 (0.98–1.08)
· Anti-S-RBD (BAU/ml)	1084.9 (970.0–1213.4)	1499.5 (1166.6–1927.4)	0.72 (0.57–0.91)
Day 90	N = 40	N = 99	
· sVNT-delta (% inhibition)	73.1 (66.7–80.2)	92.8 (90.2–95.4)	0.79 (0.73–0.85)
· sVNT-WT (% inhibition)	81.9 (76.2–88.0)	94.6 (92.4–96.9)	0.87 (0.82–0.92)
· Anti-S-RBD (BAU/ml)	744.6 (650.1–852.9)	909.0 (802.1–1030.1)	0.82 (0.66–1.02)
Day 180	N = 44		
· sVNT-delta (% inhibition)	22.7 (14.9–34.6)	–	–
· sVNT-WT (% inhibition)	40.8 (31.2–53.4)	–	–
· Anti-S-RBD (BAU/mL)	330.7 (266.3–410.8)	–	–
	% (95% CI)	% (95% CI)	% difference (95% CI)
Day 14	N = 50	N = 100	
· sVNT-delta ≥ 80% inhibition	98.0 (89.1–99.9)	93.8 (86.9–97.6)	4.2 (–2.0 to 10.5)
· sVNT-WT ≥ 80% inhibition	100.0 (92.8–100.0)	95.8 (89.7–98.9)	4.2 (0.01 to 8.2)
· Anti-S-RBD ≥ 506 BAU/ml [‡]	100.0 (92.8–100.0)	97.0 (91.5–99.4)	3.0 (–0.3 to 6.3)
Day 28	N = 50	N = 24	
· sVNT-delta ≥ 80% inhibition	98.0 (89.4–99.9)	91.7 (73.0–98.9)	6.3 (–5.4 to 18.1)
· sVNT-WT ≥ 80% inhibition	98.0 (89.4–99.9)	91.7 (73.0–98.9)	6.3 (–5.4 to 18.1)
· Anti-S-RBD ≥ 506 BAU/ml [‡]	98.0 (89.4–99.9)	96.0 (79.6–99.8)	2.0 (–6.6 to 10.6)
Day 90	N = 40	N = 99	
· sVNT-delta ≥ 80% inhibition	52.6 (35.8–69.0)	89.9 (82.2–95.0)	–37.3 (–54.2 to –20.3)
· sVNT-WT ≥ 80% inhibition	68.4 (51.3–82.5)	90.9 (83.4–95.7)	–22.5 (–38.3 to –6.7)
· Anti-S-RBD ≥ 506 BAU/ml [‡]	79.5 (63.5–90.7)	83.8 (75.1–90.5)	–4.4 (–18.9 to 10.2)
Day 180	N = 44		
· sVNT-delta ≥ 80% inhibition	11.4 (3.8–24.6)	–	–
· sVNT-WT ≥ 80% inhibition	20.4 (9.8–35.3)	–	–
· Anti-S-RBD ≥ 506 BAU/ml [‡]	29.5 (16.7–45.2)	–	–

Anti-S-RBD: Anti-spike-receptor-binding-domain, BAU: Binding-antibody unit, GM: Geometric mean, GMR: Geometric mean ratio, ID: Intradermal, IM: Intramuscular, sVNT-delta: Surrogate virus neutralization test against delta strain, sVNT-WT: Surrogate virus neutralization test against wild type.

[†] IM group from standard-dose AZD1222 booster in healthy adult completing 2-dose CoronaVac at the same study site (Chulalongkorn University, Bangkok, Thailand) of Nanthapaisal S, et al. A randomized clinical trial of a booster dose with low versus standard dose of AZD1222 in adult after 2 doses of inactivated vaccines. *Vaccine* 2022. <https://doi.org/10.1016/j.vaccine.2022.03.036>.

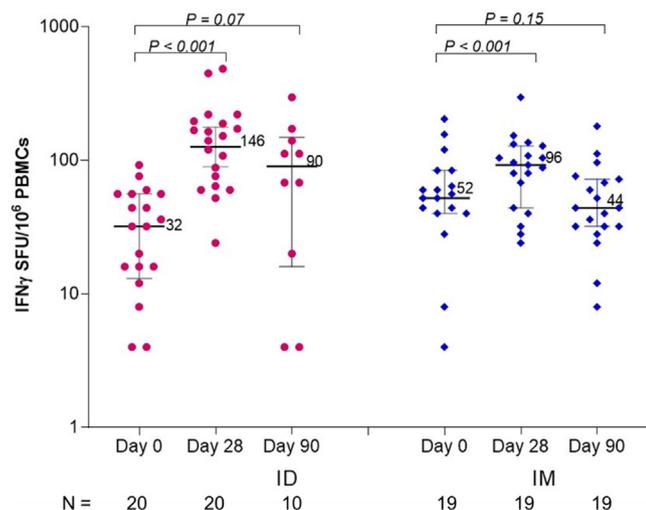
[‡] Anti-S-RBD IgG 506 BAU/ml correlated with 80% vaccine efficacy as reported by Feng S, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nature Medicine* 2021. <https://doi.org/10.1038/s41591-021-01540-1>.

compared with IM recipients, with differences of 4.2% (95% CI –2.0 to 10.5) and 6.3% (–5.4 to 18.1), respectively. Unlike at day 90, it was significantly lower, with the difference of –37.3% (–54.2 to –20.3), with lower bound of 95% CI exceeding –10%. These differences were similar to sVNT against wild type ≥ 80% inhibition and

anti-S-RBD IgG ≥ 506 BAU/ml, as shown in **Table 2**. The GMs of sVNT against delta strain post ID and IM boosters were shown in **Fig. 3**.

GMRs of sVNT against delta strain and wild type of ID boosters were non-inferior to IM boosters at all time points, as shown in

(A) SNMO-specific T cell response



(B) RBD-specific B cell response

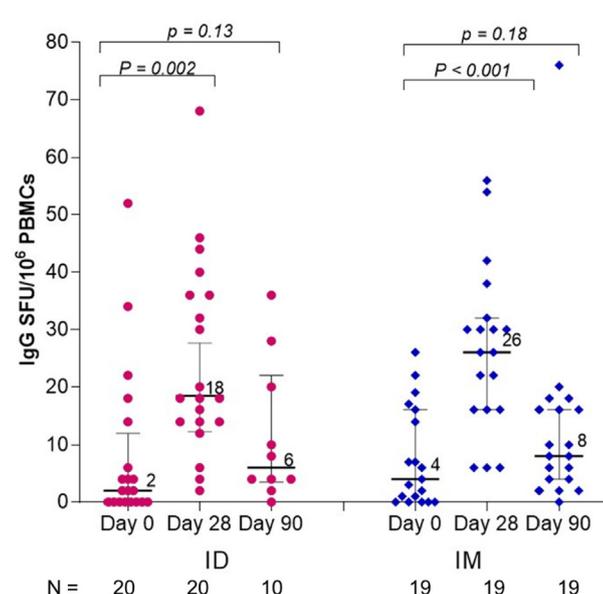


Fig. 4. ELISpot assay at day 0, day 28, and day 90 of ID and IM[†] AZD1222 booster in healthy adult completing 2-dose CoronaVac: (A) Interferon- γ ELISpot response to SNMO overlapping peptides of SARS-CoV-2, (B) RBD-specific IgG ELISpot assay. Comparison of results between day 0 and day 28 or day 90 within ID or IM group was evaluated by Wilcoxon signed rank test. Comparison of results between ID and IM group was evaluated by Wilcoxon rank sum test, yielding no statistical difference at all time points. ID: Intradermal, IM: Intramuscular, RBD: Receptor binding domain, SFU: Spot forming unit, SNMO: Spike (S) nucleoprotein (N), membrane protein (M), and open reading frame proteins (O) of SARS-CoV-2 [†]IM group from standard-dose AZD1222 booster in healthy adult completing 2-dose CoronaVac at the same study site (Chulalongkorn University, Bangkok, Thailand) of Nanthapaisal S, et al. A randomized clinical trial of a booster dose with low versus standard dose of AZD1222 in adult after 2 doses of inactivated vaccines. Vaccine 2022. <https://doi.org/10.1016/j.vaccine.2022.03.036>.

Table 2. While GMR of anti-S-RBD IgG showed non-inferiority at day 14, with GMR of 0.99 (95% CI 0.83–1.20), and borderline inferior at day 90, with GMR of 0.82 (0.66–1.02).

3.5. T And B cell responses evaluated by ELISpot assay

Participants in CMI sub study showed no different baseline characteristics, including age, sex, body mass index (BMI), comorbidities, and interval to AZD1222 boosters, to the rest of the participants. All immunogenicity parameters, including sVNT against delta strain and wild type, and anti-S-RBD IgG, at baseline, day 28, and day 90 were comparable to the others, except baseline anti-S-RBD IgG but without clinical significance (CMI sub study: median 150.5 BAU/ml, IQR 110.5–177.5; the other participants: median 97 BAU/ml, IQR 62–165; p 0.03).

From the sub study analysis of CMI response, ELISpot assay showed significant rise of T cell and B cell response at day 28 and declined at day 90, as shown in Fig. 4. Median (IQR) of IFN- γ -producing T cell spots specific to SNMO protein-derived peptide pools at day 0 was 32 (14–56), at day 28 was 146 (70–192), at day 90 was 90 (20–140), and at day 180 was 56 (36–88) SFU/10⁶ PBMCs, respectively. Compared with IM study, the median (IQR) was 52 (40–48) at day 0, 96 (44–128) at day 28, and 44 (32–72) SFU/10⁶ PBMCs at day 90, respectively. Median (IQR) of RBD-specific memory B cell spots at day 0 was 2 (0–10), increased to 18 (14–36) at day 28, declined to 6 (4–20) at day 90, and 0 (0–65) SFU/10⁶ PBMCs at day 180. Compared with IM study of 4 (0–16) at day 0, 26 (16–32) at day 28, and 8 (4–16) SFU/10⁶ PBMCs at day 90.

3.6. Immunogenicity of ID AZD1222 booster during omicron predominance

One participant acquired COVID-19 after ID booster. A 27-year-old Thai male got COVID-19 upper respiratory illness at 127 days post ID booster, during December 2021, and recovered uneventfully. While there was no COVID-19 case in IM booster group, which was followed up during September to November 2021, the period of delta variant predominance.

4. Discussion

ID AZD1222 booster vaccine in 2-dose-CoronaVac-primed adults raised high anti-S-RBD IgG greater than 506 BAU/ml, and high levels of functional neutralizing antibodies greater than 80% inhibition as measured by sVNT to wild type and delta strain, thus non-inferior to IM route at day 14. However, at 3 months post ID AZD1222 booster vaccination, this study demonstrated, despite similar anti-S-RBD IgG, but lower sVNT against delta strain to IM booster, suggesting more rapid waning neutralizing antibody response after ID compared to IM route. Most reactogenicity occurred locally with erythema, pain, and swelling at injection site. Erythema, swelling, and blister were reported more common in ID booster. Systemic symptoms such as fever, feverish, headache, fatigue, and myalgia were less common than conventional IM injection.

The non-inferior immunogenicity of ID vaccination was demonstrated for influenza, rabies, and hepatitis B vaccines [21]. Immunogenicity of ID AZD1222 at day 14 was not inferior to conventional IM booster vaccine as shown with difference in proportion of participants having sVNT to delta strain and wild type passing 80% and GMR of anti-S-RBD. This comparable result is similar to previous study in Netherland [13] which reported a robust antibody response from ID administration of mRNA COVID-19 vaccine at day 43 with comparable anti-spike IgG response for 10 μ g ID with 100 μ g IM mRNA-1273 vaccine. Additionally, recent report from Thailand also denoted a fractional-dose BNT162b2 ID booster, in healthy adults who had completed 2-dose inactivated vaccine

for 2–3 months, induced comparable antibody level and function to the conventional IM booster when assessed on day 14 and 28 [14]. To our knowledge, no published report demonstrated immunogenicity results at 3 months post ID AZD1222 booster vaccination, which this study demonstrated inferior neutralizing antibodies.

Although, the importance of cellular immunity in correlation with vaccine protection is still unclear, specific T cells have been reported to reduce the severity of SARS-CoV-2 infection [22]. In this study, we have shown that a third dose of ID AZD1222 booster vaccine could increase specific T cell responses slightly higher than conventional IM route, similar to the previous report [23]. As opposed to previous ID BNT162b2 study, which failed to demonstrate T cell response, suggesting different vaccine platforms might play a role in cellular immune response. Specific memory B cells also have been reported to play a crucial role for effective responses to infection [24,25]. Our result showed the slight boost of B cell response at 1 month and drop at 3 months, after ID AZD1222 booster. The timing of B cell study might be accounted for these responses since the previous study showed the detectable B cell response after COVID-19 infection for 3–6 months [26].

This study reported local reactogenicity including erythema, blister, and pruritus after ID AZD1222 booster vaccine which is similar to previous report on rabies inactivated vaccine that more erythema and pruritus were reported from ID than conventional IM administration [27]. Also blister formation was reported after BCG vaccination that evolved over two weeks into an ulcer at injection site [28]. ID influenza vaccine study reported significant higher local adverse events particularly erythema and swelling, and also more common of fever and chills which is different from this study that fever was more common in IM vaccination [9]. Hyperpigmentation was also reported in this study as still seen on day 28 of follow-up visit, similar to previous report of local hyperpigmentation after hepatitis B ID vaccination in 55% [29]. Compared with parallel IM study, ID booster had more local reactogenicity (erythema and swelling) at injection site but less pain and systemic reactogenicity (fatigue, myalgia, headache, feverish, arthralgia and diarrhea), which were acceptable in the participants. AZD1222 or ChAdOx1 nCoV-19 vaccine consists of the replication-deficient adenoviral vector ChAdOx1 containing spike protein of SARS-CoV-2 [30], without any adjuvants. The use of adjuvants, components capable of enhancing and/or shaping antigen-specific immune responses [31], might improve the immunogenicity of intradermal vaccine delivery, although might lead to more local reactions.

As current situation of COVID-19 pandemic, more vaccine supply is still needed for many countries as vaccine coverage is not enough to prevent mortality [32–34]. Almost half of the world population have received at least one dose of COVID-19 vaccine but accounted for only 2.2% of people in low-income countries [35]. AZD1222 or AstraZeneca/Oxford COVID-19 vaccine has been used in Europe since December 2020 and distributed in many countries including low- to middle-income countries [36]. As availability in many countries with limited vaccine supply, the ID administration of AZD1222 might be considered for mass vaccination as an advantage of dose-sparing technique [7]. However, there are some limitations, needs of skilled health providers for administration [37], and more rapid waning of neutralizing antibodies.

Intradermal vaccination by Mantoux technique was shown to induce similar immune response to standard route using lower dose, likely due to the abundance of immune cells in dermis [16]. However, this manual technique requires expertise to perform it correctly. In Thailand, ID administration of rabies vaccines has been implemented as a choice for post-exposure prophylaxis [12], leading to widely acceptance and expertise of health personnel to perform manual ID vaccination. Apart from manual tech-

nique, several devices and techniques have been studied in various stages of development, e.g., needle adapter, jet and ballistic delivery, thermal ablation, etc., which offered easier application, though specific devices are required [8], precluding them from vast implementation, especially in developing countries.

This study was limited by cohort study design without randomized controlled trial but there was the parallel cohort study with similar settings that should be able to benchmark the results. There were 2 factors that differed between the 2 groups. Specifically, there was more male in the ID group and the time interval between second and third dose was 1 week longer in the ID group. However, the immune responses at baseline before the third dose were comparable. Female was reported to have higher antibody response to vaccines [38] and after severe COVID-19 [39]. The finding of later inferior neutralizing antibodies might be attributed to this gender difference, specifically more male participants in ID cohort, and the one-fifth dose might be too low to provide comparable immunogenicity. This study chose to determine the levels of functional neutralizing antibodies using the surrogate virus neutralization assay, rather than standard live-virus neutralization assay. However, we used the high cut-off value at 80% of sVNT in this study. Moreover, good correlations between sVNT and live-virus neutralization have been exhibited elsewhere [19,40–42]. The other limitation is the different follow-up time, 6 months for ID vs 3 months for IM, resulting in no data on COVID-19 breakthrough infection during the omicron era in the IM cohort. The strengths of this study were reporting complete solicited reactogenicity of all 100 participants with ID booster vaccination and multiple methods used for immunity analysis including anti-S-RBD, sVNT (wild type and delta strain) and also CMI responses.

ID AZD1222 booster vaccine in 2-dose-CoronaVac primed adult enhanced comparable short-term neutralizing antibodies, but inferior at 3 months, with intramuscular administration. T and B cell-mediated immune responses were boosted after ID booster, similar to IM route. Reactogenicity was usually localized (erythema and pain) and less systemic than intramuscular vaccine. Due to more rapid waning neutralizing antibody, dose-sparing strategy with intradermal booster vaccination should be used in the setting of inadequate vaccine supply. Fractional doses of COVID-19 vaccines could speed up vaccine coverages and save lives, even with lower efficacy, especially in the era of omicron pandemic [43].

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.

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Appendix A. Supplementary material

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References

- [1] World Health Organization. WHO Coronavirus (COVID-19) Dashboard [Available from: <https://covid19.who.int/>]. Accessed date 28 November, 2021.
- [2] Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med* 2021;385(10):875–84.
- [3] Hitchings MDT, Ranzani OT, Torres MSS, de Oliveira SB, Almiron M, Said R, et al. Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: A test-negative case-control study. *Lancet Reg Health Am* 2021;1:100025.
- [4] Vacharathit V, Aiewsakun P, Manopwisedjaroen S, Srisaowakarn C, Laopanupong T, Ludowyke N, et al. CoronaVac induces lower neutralising activity against variants of concern than natural infection. *Lancet Infect Dis* 2021;21(10):1352–4.
- [5] Liu X, Shaw RH, Stuart ASV, Greenland M, Aley PK, Andrews NJ, et al. Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial. *Lancet* 2021;398(10303):856–69.
- [6] Benning L, Tollner M, Hidmark A, Schaijer M, Nusshag C, Kalble F, et al. Heterologous ChAdOx1 nCoV-19/BNT162b2 Prime-Boost Vaccination Induces Strong Humoral Responses among Health Care Workers. *Vaccines (Basel)* 2021;9. <https://doi.org/10.3390/vaccines9080857>.
- [7] Migliore A, Gigliucci G, Di Marzo R, Russo D, Mammucari M. Intradermal Vaccination: A Potential Tool in the Battle Against the COVID-19 Pandemic? *Risk Manag Healthc Policy* 2021;14:2079–87. <https://doi.org/10.2147/RMHP.S309707>.
- [8] Hettaing J, Carlisle R. Vaccination into the Dermal Compartment: Techniques, Challenges, and Prospects. *Vaccines (Basel)* 2020;8(3):534.
- [9] Egunsola O, Clement F, Taplin J, Mastikhina L, Li JW, Lorenzetti DL, et al. Immunogenicity and Safety of Reduced-Dose Intradermal vs Intramuscular Influenza Vaccines: A Systematic Review and Meta-analysis. *JAMA Netw Open* 2021;4(2):e2035693.
- [10] World Health Organization. Polio vaccines: WHO position paper, March 2016-recommendations. *Vaccine* 2017;35:1197–9. <https://doi.org/10.1016/j.vaccine.2016.11.017>.
- [11] World Health Organization. WHO Expert Consultation on Rabies. Second report. *World Health Organ Tech Rep Ser* 2013;1:1–139, back cover.
- [12] World Health Organization. Rabies vaccines: WHO position paper, April 2018 - Recommendations. *Vaccine* 2018;36:5500–3. <https://doi.org/10.1016/j.vaccine.2018.06.061>.
- [13] Rozen GVT, Prins MLM, van Binnendijk R, Hartog Gd, Kuiper VP, Prins C, et al. Tolerability, safety and immunogenicity of intradermal delivery of a fractional dose mRNA-1273 SARS-CoV-2 vaccine in healthy adults as a dose sparing strategy. *medRxiv* 2021:2021.07.27.21261116. <https://doi.org/10.1101/2021.07.27.21261116>.
- [14] Intapiboon P, Seepathomnarong P, Ongarj J, Surasombatpattana S, Uppanisakorn S, Mahasirimongkol S, et al. Immunogenicity and Safety of an Intradermal BNT162b2 mRNA Vaccine Booster after Two Doses of Inactivated SARS-CoV-2 Vaccine in Healthy Population. *Vaccines* 2021;9(12):1375.
- [15] Singhatiraj E, Pongpirul K, Jongkaewwattana A, Hirankarn N. Intradermal ChAdOx1 Vaccine Following Two CoronaVac Shots: A Case Report. *Vaccines* 2021;9:990.
- [16] Lambert PH, Laurent PE. Intradermal vaccine delivery: will new delivery systems transform vaccine administration? *Vaccine* 2008;26:3197–208. <https://doi.org/10.1016/j.vaccine.2008.03.095>.
- [17] Amanat F, Stadlbauer D, Strohmaier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med* 2020;26(7):1033–6.
- [18] Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med* 2021;27(11):2032–40.
- [19] Tan CW, Chia WN, Qin X, Liu P, Chen M-C, Tiu C, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nat Biotechnol* 2020;38(9):1073–8.
- [20] U.S. Department of Health and Human Services (FDA). Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials September 2007 [Available from: <https://www.fda.gov/media/73679/download>]. Accessed date 30 November, 2021.
- [21] Schnyder JL, De Pijper CA, Garcia Garrido HM, Daams JG, Goorhuis A, Stijnis C, et al. Fractional dose of intradermal compared to intramuscular and subcutaneous vaccination - A systematic review and meta-analysis. *Travel Med Infect Dis* 2020;37:101868.
- [22] Noh JY, Jeong HW, Kim JH, Shin E-C. T cell-oriented strategies for controlling the COVID-19 pandemic. *Nat Rev Immunol* 2021;21(11):687–8.
- [23] Rosenbaum P, Tchitchek N, Joly C, Rodriguez Pozo A, Stimmer L, Langlois S, et al. Vaccine Inoculation Route Modulates Early Immunity and Consequently Antigen-Specific Immune Response. *Front Immunol* 2021;12. <https://doi.org/10.3389/fimmu.2021.645210645210>.
- [24] Jahnmatz P, Sundling C, Makower B, Sonden K, Farnert A, Ahlborg N. Multiplex analysis of antigen-specific memory B cells in humans using reversed B-cell FluoroSpot. *J Immunol Methods* 2020;478. <https://doi.org/10.1016/j.jim.2019.112715112715>.
- [25] Ciabattini A, Pastore G, Fiorino F, Polvere J, Lucchesi S, Pettini E, et al. Evidence of SARS-CoV-2-Specific Memory B Cells Six Months After Vaccination With the BNT162b2 mRNA Vaccine. *Front Immunol* 2021;12. <https://doi.org/10.3389/fimmu.2021.740708740708>.
- [26] Sherina N, Piralla A, Du L, Wan H, Kumagai-Braesch M, Andrej J, et al. Persistence of SARS-CoV-2-specific B and T cell responses in convalescent COVID-19 patients 6–8 months after the infection. *Med (N Y)* 2021;2(281–95). <https://doi.org/10.1016/j.medi.2021.02.001e4>.
- [27] Angsuwatcharakon P, Ratananpinit N, Yoksan S, Saengseesom W, Sriksorn R, Raksahket N, et al. Immunogenicity and safety of two-visit, intradermal pre-exposure rabies prophylaxis simultaneously administered with chimeric live-attenuated Japanese encephalitis vaccine in children living in rabies and Japanese encephalitis endemic country. *Vaccine* 2020;38(32):5015–20.
- [28] Fritz TM, Trueb RM. BCG ulcer. Rapid healing with topical INH powder. *Hautarzt* 2002;53:816–8. <https://doi.org/10.1007/s00105-002-0367-3>.
- [29] Lankarani KB, Taghavi AR, Agah S, Karimi A. Comparison of intradermal and intramuscular administration of hepatitis B vaccine in neonates. *Indian J Gastroenterol* 2001;20:94–6.
- [30] Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020;396(10249):467–78.
- [31] Reed SC, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. *Nat Med* 2013;19:1597–608. <https://doi.org/10.1038/nm.3409>.
- [32] Blanchet K, Mallard G, Moret E, Sun J. Sanctioned countries in the global COVID-19 vaccination campaign: the forgotten 70. *Confl Health* 2021;15:69. <https://doi.org/10.1186/s13031-021-00404-2>.
- [33] Perry M, Akbari A, Cottrell S, Gravenor MB, Roberts R, Lyons RA, et al. Inequalities in coverage of COVID-19 vaccination: A population register based cross-sectional study in Wales. *UK Vaccine* 2021;39(4):6256–61.
- [34] Kupek E. Low COVID-19 vaccination coverage and high COVID-19 mortality rates in Brazilian elderly. *Rev Bras Epidemiol* 2021;24. <https://doi.org/10.1590/1980-549720210041e210041>.
- [35] Hannah Ritchie EM, Lucas Rodés-Guirao, Cameron Appel, Charlie Giattino, Esteban Ortiz-Ospina, Joe Hasell, Bobbie Macdonald, Diana Beltekian and Max Roser. Coronavirus Pandemic (COVID-19): Our World in Data; 2020 [cited 2021 26 sep 2021]. Available from: <https://ourworldindata.org/covid-vaccinations>. Accessed date 26 September, 2021.
- [36] Ghasemiyeh P, Mohammadi-Samani S, Firouzabadi N, Dehshahri A, Vazin A. A focused review on technologies, mechanisms, safety, and efficacy of available COVID-19 vaccines. *Int Immunopharmacol* 2021;100. <https://doi.org/10.1016/j.intimp.2021.108162108162>.
- [37] Schweiger M. Intradermal covid-19 vaccination could solve supply problems. *BMJ* 2021;374. <https://doi.org/10.1136/bmj.n1980n1980>.
- [38] Ciarambino T, Para O, Giordano M. Immune system and COVID-19 by sex differences and age. *Womens Health (Lond)* 2021;17:17455065211022262. <https://doi.org/10.1177/17455065211022262>.
- [39] Zeng F, Dai C, Cai P, Wang J, Xu L, Li J, et al. A comparison study of SARS-CoV-2 IgG antibody between male and female COVID-19 patients: A possible reason underlying different outcome between sex. *J Med Virol* 2020;92(10):2050–4.
- [40] Abe KT, Li Z, Samson R, Samavarchi-Tehrani P, Valcuro EJ, Wood H, et al. A simple protein-based surrogate neutralization assay for SARS-CoV-2. *JCI Insight* 2020;5. <https://doi.org/10.1172/jci.insight.142362>.

- [41] Meyer B, Reimerink J, Torriani G, Brouwer F, Godeke GJ, Yerly S, et al. Validation and clinical evaluation of a SARS-CoV-2 surrogate virus neutralisation test (sVNT). *Emerg Microbes Infect* 2020;9:2394–403. <https://doi.org/10.1080/22221751.2020.1835448>.
- [42] Valcourt EJ, Manguiat K, Robinson A, Chen J-Y, Dimitrova K, Philipson C, et al. Evaluation of a commercially-available surrogate virus neutralization test for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *Diagn Microbiol Infect Dis* 2021;99(4):115294.
- [43] Więcek W, Ahuja A, Chaudhuri E, Kremer M, Simoes Gomes A, Snyder CM, et al. Testing fractional doses of COVID-19 vaccines. *Proc Natl Acad Sci U S A* 2022;119(8). <https://doi.org/10.1073/pnas.2116932119>.