Study Protocol

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Co-administration of COVID-19 and Influenza Vaccines: A Pilot Randomized Controlled Trial in Healthy Adults

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Background

Coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) in March 2020, owing to its rapid global spread and the resulting high rates of illness and death. In May 2023, the WHO indicated that the COVID-19 public health emergency of international concern had concluded, officially marking the end of the pandemic [1]. However, COVID-19 remains a persistent disease and is now transitioning toward an endemic status [1]. Control of COVID-19 transmission was achieved through herd immunity from vaccination or previous infection. The Centers for Disease Control and Prevention advises annual updates for both COVID-19 and flu vaccinations [2]. Recent studies suggest that the influenza vaccine may help reduce COVID-19 infection rates and related deaths [3–6]. Analyses at the national level in the United States have shown an inverse relationship between flu vaccine coverage in older adults and COVID-19 deaths [7]; similar results were observed in Italy [8]. The protective effect could be explained by cross-reactive immunity, which is mediated by memory T-cells triggering both cell-mediated immune response (CMIR) and humoral immune response (HIR) [9]. A previous study identified cross-reactive antibodies between glycan epitopes of the COVID-19 virus spike protein and the influenza virus surface protein. Although non-neutralizing antibodies, they may facilitate antibody-dependent cellular cytotoxicity [10]. Another proposed mechanism involves the broad immunological effects of vaccines, which enhance trained immunity by modifying the gene expression patterns of innate immune cells [11]. A previous study reported markedly higher immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralization titers among workers vaccinated with both flu and pneumococcal vaccines or with the flu vaccine alone [12]. Another study found higher levels of anti-spike receptor-binding domain (RBD) antibodies in individuals who received the flu vaccine, in contrast to those who were unvaccinated before receiving the BNT162b2 vaccine [13].

Additionally, six randomized controlled trials (RCTs) examined the safety or immunogenicity of administering mRNA COVID-19 doses alongside influenza vaccines [14–19]. All these studies have confirmed the safety of administering the vaccines together; however, the findings on immunogenicity were mixed. Out of the six RCTs, five evaluated HIR and observed reduced anti-SARS-CoV-2 antibody concentrations in those receiving both vaccines [15–19]. Only two studies met the non-inferiority margin for immunogenicity outcomes [15, 18]. However, none have yet evaluated CMIR.

Owing to the lack of prior research evaluating CMIR, we conducted a pilot study to assess both HIR and CMIR for COVID-19 and influenza vaccines administered together compared with individually administering each vaccine. We hypothesized that the CMIR may be stronger in the co-administration group, which could help explain the reduced mortality from COVID-19 infections reported in previous studies for patients vaccinated with the influenza vaccine. If confirmed, a stronger CMIR may serve as a proof-of-concept, demonstrating the potential immunological benefits of vaccine co-administration. Additionally, our study may provide insightful findings on the co-administration policy, the implementation of which may increase vaccine coverage and reduce healthcare burden.

Objectives

Primary Objective:

- To evaluate and compare post-vaccination cell-mediated immune responses (CMIR) and humoral immune responses (HIR) between participants receiving co-administration of COVID-19 and influenza vaccines versus COVID-19 vaccine alone.

Secondary Objectives:

- To assess the magnitude of immune responses within each study group.
- To compare the magnitude of immune enhancement between groups.
- To evaluate the frequency and severity of adverse events following vaccination.

Study Design and Participants

This was a pilot, single-blind, randomized controlled trial conducted at Songklanagarind Hospital, Faculty of Medicine, Prince of Songkla University, Hat Yai, Thailand. Eligible participants were healthy adult volunteers aged 18–60 years.

Inclusion Criteria

- Thai adults aged 18–60 years who had received COVID-19 and influenza vaccines more than 6 months prior.
- Able and willing to comply with trial requirements, including 1-month follow-up.
- In good health at enrollment as judged by medical history, physical exam, and investigator assessment.
- Able to provide informed consent and sign the ICF.

Exclusion Criteria

- History of influenza or COVID-19 infection within 6 months.
- History or family history of convulsions, epilepsy, encephalopathy, or psychosis.
- Allergy to vaccine components or prior severe hypersensitivity reactions.
- History of Guillain-Barré syndrome.
- Positive pregnancy test, pregnant, breastfeeding, or pregnancy plan within 6 months.
- Infectious diseases including HIV or suspected SARS-CoV-2 infection.
- Prior SARS-CoV-2 infection within 6 months.
- Severe uncontrolled chronic diseases (e.g., poorly controlled diabetes mellitus, uncontrolled hypertension).
- History of urticaria within 1 year before vaccination.
- Known coagulation disorders or thrombocytopenia contraindicating intramuscular injection.
- Needle phobia.
- Use of immunosuppressive therapy, cytotoxic therapy, or systemic corticosteroids.
- Receipt of blood products within 4 months before vaccination.

- Ongoing anti-tuberculosis treatment.
- Inability to follow protocol or understand informed consent, as judged by investigator.

Interventions

Arm 1 – Active Comparator: Bivalent mRNA COVID-19 vaccine (BNT162b2)

- Intervention: Biological/Vaccine – Bivalent mRNA SARS-CoV-2 vaccine
- Description: Administering a booster dose of the bivalent mRNA vaccine BNT162b2 (Pfizer, Bangkok, Thailand).

Arm 2 – Experimental: Quadrivalent Influenza Vaccine

- Intervention: Biological/Vaccine – Quadrivalent influenza vaccine
- Description: Administering a VaxigripTetra™ (Sanofi-Aventis, Bangkok, Thailand).

Arm 3 – Experimental: Co-administration

- Interventions:
  - Biological/Vaccine – Bivalent mRNA SARS-CoV-2 vaccine (BNT162b2)
  - Biological/Vaccine – Quadrivalent influenza vaccine (VaxigripTetra™)
- Description: Co-administering both vaccines intramuscularly, either in different arms or in the same arm at least 25.4 mm apart.

Outcomes

Primary Outcomes

- **Cell-mediated immune response (CMIR):** Post-vaccination interferon-gamma enzyme-linked immunospot (IFN-γ ELISpot) assay results comparing the co-administration group with the COVID-19 vaccine–only group.
- **Humoral immune response (HIR):** Post-vaccination anti-receptor-binding domain immunoglobulin G (anti-RBD IgG) concentrations comparing the co-administration group with the COVID-19 vaccine–only group.
- **Non-inferiority margin:** Defined as the lower bound of the 95% confidence interval for the geometric mean ratio (GMR) >0.67, consistent with WHO vaccine evaluation guidelines.

Secondary Outcomes

- **Magnitude of immune response within each group:** GMR comparing pre- and post-vaccination values (GMRpost/pre) for both CMIR and HIR.
- **Comparison of immune enhancement between groups:** Ratio of GMRpost/pre values across groups.
- **Adverse events (AEs):** Incidence, type, and severity of local and systemic solicited AEs at 1 and 4 weeks, as well as unsolicited and serious AEs.

Study Procedures

Eligible volunteers provided written informed consent before participation. Participants were randomized in a 1:1:1 ratio, using block randomization (block size of 6) generated with R Statistical Software, to receive:

1. Bivalent mRNA COVID-19 vaccine (BNT162b2, Pfizer) alone,
2. Quadrivalent influenza vaccine (VaxigripTetra™, Sanofi-Aventis) alone, or
3. Both vaccines administered concurrently.

Vaccines were administered intramuscularly, either in different arms or in the same arm with injection sites at least 25.4 mm apart.

Blood samples were collected at baseline (Day 0, prior to vaccination) and at Day 28. Samples were used for measurement of:

- Humoral immune response (HIR): anti-receptor binding domain (RBD) IgG levels.
- Cell-mediated immune response (CMIR): IFN-γ enzyme-linked immunospot (ELISpot) assay.

Participants were monitored for immediate adverse reactions for 30 minutes after vaccination. Active follow-up was conducted at 1- and 4-weeks post-vaccination to assess solicited local and systemic adverse events, and unsolicited or serious adverse events were documented throughout the study period.

Sample Size

A total of 36 participants was planned. The sample size followed Julious' rule-of-thumb[20] for pilot studies (≥12 per group) to obtain stable variance estimates and inform the design of a subsequent adequately powered non-inferiority RCT. The study was not powered for confirmatory hypothesis testing; non-inferiority analyses are exploratory. Estimates from this pilot (means, variances, GMRs) will guide the definitive trial's sample-size calculation.

Statistical Methods

Analysis Population

- All randomized participants who received at least one dose of study vaccine were included in the immunogenicity and safety analyses (modified intention-to-treat).

Data Handling and Transformation

- Immune response outcomes (IFN-γ ELISpot and anti-RBD IgG antibody levels) were log-transformed prior to analysis to normalize distributions and stabilize variance.
- Zero values were replaced with 1 to allow logarithmic transformation.

Primary Analysis

- Geometric mean ratios (GMRs) of post-vaccination responses were calculated to compare the co-administration group with the COVID-19 vaccine–only group.
- Non-inferiority was concluded if the lower bound of the two-sided 95% confidence interval (CI) exceeded **0.67**, consistent with WHO vaccine evaluation guidelines.

Secondary Analyses

1. **Magnitude of immune response (within-group):**
    - Calculated as GMRpost/pre for both CMIR and HIR.
    - Paired t-tests were used to assess the significance of pre- vs post-vaccination changes.
2. **Between-group comparisons (ratio of magnitude):**
    - Ratios of GMRpost/pre between study groups were calculated to compare immune enhancement.
3. **Safety analysis:**
    - Incidences of solicited local and systemic adverse events were summarized by frequency and percentage.
    - Group comparisons used Chi-square or Fisher's exact tests, as appropriate.

Software

- All statistical analyses were conducted using **R Statistical Software, version 4.3.2** (R Foundation for Statistical Computing, Vienna, Austria).

Laboratory Methods

- **Antibody analysis:** Anti–SARS-CoV-2 RBD IgG (Wuhan-Hu-1 strain) will be measured by chemiluminescent microparticle immunoassay (Abbott, Abbott Park, IL, USA). Results in arbitrary units (AU/mL) will be converted to WHO binding antibody units (BAU/mL) using 1 BAU/mL = 0.142 AU/mL.

- **Ex vivo IFN-γ ELISpot assays:** Peripheral blood mononuclear cells (PBMCs) will be stimulated with SARS-CoV-2 spike peptide pools (Wuhan strain) and assessed in triplicate wells. Spot-forming cells (SFCs) per 10^6 PBMCs will be quantified using an automated ELISpot counter (AID Diagnostika, Konstanz, Germany), subtracting background from negative controls.

Adverse Event Monitoring

All participants were observed on-site for 30 minutes post-vaccination to detect any immediate reactions.

Solicited Adverse Events (AEs):

- Local AEs: pain, redness, swelling, and palpable mass at the injection site.
- Systemic AEs: fever, myalgia, malaise, headache, nausea, and other constitutional symptoms.

- These pre-specified solicited AEs were actively assessed at 1 week and 4 weeks after vaccination.

Unsolicited and Serious Adverse Events (SAEs):

- Any unsolicited AE reported during the study period was documented.
- SAEs were recorded throughout the follow-up period, with severity grading and assessment of relationship to study vaccines.

Ethics and Dissemination

The study protocol was reviewed and approved by the Human Research Ethics Committee of Prince of Songkla University (REC. 66-462-14-1; approval date: 2 January 2024). The trial was conducted in accordance with the principles of the Declaration of Helsinki and applicable Good Clinical Practice (GCP) guidelines.

All participants provided written informed consent before enrollment. Confidentiality of participant data was strictly maintained, and only de-identified data were used for analysis.

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