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|  | Statistical Analysis Plan | | | | |
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| |  | | --- | | **Title of Proposed Research (with subtitle specifying the design, e.g., case-control)** | | Tuberculosis following two-dose SARS-CoV-2 vaccination with messenger RNA vaccine (BNT162b2) and inactivated virus vaccine (CoronaVac): a retrospective cohort study  Protocol version: version 1  Patients: Hospital Authority active users  Intervention: two-dose SARS-CoV-2 vaccination  Control: unvaccinated individuals by Sep 30, 2021  Outcome: Incident TB diagnosed from inpatient settings | | | | | | |
| title page | | | | | |
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| Publicly available on CARE website? <https://www.hkcare.hku.hk/> | | Yes  Date: | | May 27, 2022 | No |

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| 1. | BACKGROUND |
|  | The ongoing COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to more than 500 million cases and 6 million deaths1. Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is the second leading cause of infectious death compared with COVID-192. Bacille Calmette-Guerin (BCG) vaccine, an attenuated live vaccine used to protect against tuberculosis for over 100 years, may protect against COVID-19 and reduce related mortality by inducing trained immunity according to the latest epidemiological studies and animal studies3-5. Trained immunity is a form of adaptation of innate host defence mechanisms or a *de facto* innate immune memory. Following exposure to particular infectious agents or vaccines, trained immunity can mount a faster and greater response against a secondary challenge with the same or even different pathogens.6,7 Unlike adaptive immunity, trained immunity does not rely on antigen-specificity and therefore was linked to cross-pathogen-protection effects. The protective effect of the non-mycobacterium infection of BCG vaccine has been found on staphylococci, candidiasis, yellow fever, and influenza by increasing the level of IFN-γ, TNF, and IL-1β8,9. Cross-pathogen protection has also been reported for the influenza vaccine. A retrospective cohort study10 suggested influenza vaccination may protect against TB, the potential mechanism may be due to activation of T-helper 17 (Th17) lymphocytes, a subgroup of lymphocytes characterized by secretion of IL-1711,12. Increased Th-17 response has also been identified with SARS-CoV-2 vaccination13,14. Enlightened by the TB protection effect from BCG and influenza vaccines, we hypothesize that SARS-CoV-2 vaccination could also trigger trained immunity and offer protection against tuberculosis in return. |
| 2. | [OBJECTIVES](#_Toc351646345) |
|  | To investigate the effect of two-dose SARS-CoV-2 vaccination on the occurrence of TB. |
| 3. | [STUDY](#_Toc351646344) DESIGN |
| 3a | Design: Retrospective cohort study |
| 3b | Data source:Hospital Authority (HA) electronic health records (EMR) database:The HA is a statutory body managing 43 hospitals and institutions, 49 Specialist Out-patient Clinics (SOPC), and 73 General Out-patient Clinics (GOPC) in Hong Kong and provides healthcare services to all of the 7 million HK residents15. The anonymised EHRs include information about demographics, death, diagnoses, drug prescription records, hospitalisation, attendances, and laboratory tests16Periods covered: 01/01/2018 to 01/31/2022 Department of Health (DH) vaccination records database: The database consists of date of vaccination, vaccination sequence, vaccine brand, and recipient’s baseline information Periods covered: 02/23/2021 to 01/31/2022 The record-linked EMR database has been used for several population-based pharmacovigilance studies for the SARS-CoV-2 vaccine with proven population representativeness and data accuracy17-32 |
| 3c | Details of data handling:  Two-dose vaccine recipients (need to complete two-dose vaccination on or before Sep 30, 2021, to allow adequate follow-up time) were matched with unvaccinated individuals by density matching (By age and sex). Pseudo vaccination date will be assigned to unvaccinated individuals accordingly.   * Exclude subjects whose registered death before pseudo index date of 2nd dose * Exclude subjects with metastatic cancer before date of 1st dose * Exclude subjects with TB diagnosis history (any setting) and prescription history (Isoniazid or rifampin**)** before the date of 1st dose * Exclude subjects with 2 different brands of vaccine * Exclude subjects aged <18 years * Exclude subjects who had first dose before Sep 30, 2021, and second dose after (i.e., partial vaccination) |
| 4. | STUDY POPULATION |
|  | Hospital Authority active users |
| 5 | STUDY OUTCOME |
| 5a | Primary outcome: Newly diagnosed TB cases (ICD-9-CM: 010-018) in inpatient setting primary ranking verified by isoniazid + rifampin + pyrazinamide + [ethambutol or streptomycin)] within 14 days after admission |
| 5b | Secondary outcomes: Newly diagnosed TB cases (ICD-9-CM: 010-018) in inpatient setting primary ranking. |
| 6 | EXPOSURE |
| 6a | Primary exposure: Two-dose Covid-19 vaccination (BNT162b2 or CoronaVac) |
| 6b | Secondary exposure: NA |
| 7 | CONFOUNDERS/COVARIATES |
|  | Charlson comorbidity index (CCI), non-CCI medical conditions including stroke or systemic embolism, asthma, respiratory infections, viral infections, systemic lupus erythematosus, psoriatic arthritis, spondylarthrosis, multiple sclerosis, inflammatory bowel disease; Recent one-year healthcare resource utilization: number of IP admission, number of AE admission, number of SOPC visits, number of GOPC visits; Covid-19 infection before admission date defined as one positive result on the SARS–CoV–2 polymerase chain reaction (PCR) test; Prescriptions within 180 days to index date, binary variable: antiviral drugs; antibiotics; steroid; immunosuppressants; anticoagulants; antiplatelet medications; beta blocker; calcium channel blockers; statin; angiotensin-converting enzyme; angiotensin receptor blockers; digoxin. ICD-9 codes used to identify diagnosis and clinical history and drug name, British National Formulary (BNF) code used to identify prescription were detailed in supplementary table 1 and supplementary table 2 |
| 8 | EFFECT MODIFICATION/STRATIFICATION |
|  | Subgroup analyses will be conducted by gender and age group (18-59, 60+), additionally, we will add 30-day washout period after 2nd dose to ascertain the level of T-cell response |
| 9 | ANALYSIS |
| 9a | Main analysis: Multi-group Inverse Probability of Treatment Weighting (IPTW) will be used to balance of patient characteristics across three groups. Weighted results will be trimmed by 1% of extreme values (two-sided). Cox Proportional-Hazards model will be applied to estimate the hazard ratio (HR) of incident TB using the unvaccinated group as reference, adjusted by variables with the standard mean difference greater than 0.1 after IPTW. Proportional hazards assumption will be tested using scaled Schoenfeld residuals. |
| 9b | Sub-group analysis: By gender and age group (18-59, 60+) |
| 9c | Sensitivity analysis:Adding 30-day washout period after 2nd dose (Only consider TB occurred 30 days after 2nd dose) to test the impact of different level of T-cell response  * Appendicitis (ICD-9 diagnostic codes: 540-543, procedure code 47.x) as a negative outcome control to detect potential selection bias33 * Using Fine-Gray regression to adjust the effect of competing risk from all-cause death34 |
| 10 | ANTICIPATED PITFALLS |
|  | 1. TB patients may visit private hospital thus not captured in our study 2. Disease and prescription history could look back to 2018 to define the incident TB. This would not exclude the possibility that the TB is re-active latent TB as the incubation time of TB can be decades. 3. Key assumption of Cox regression is the effect of intervention is constant over study period, which is likely to be violated in our study as vaccine efficacy is not constant. We will test the assumption and plot the trend of hazard ratios by time to detect time-varying effect. |
| 11 | RELEVANT RESEARCH CHECKLIST |
|  | STrengthening the Reporting of OBservational studies in Epidemiology for Cohort study |
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**Supplementary Table 1** **ICD-9 codes used to identify diagnosis and clinical history**

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| Description | ICD9 codes |
| **Charlson Comorbidity Index** |  |
| Myocardial infarction | 410 |
| Peripheral vascular disease | 441, 443.9, 785.4, V43.4 |
| Cerebrovascular disease | 430-438 |
| Chronic obstructive pulmonary disease | 490-496, 500-505, 506.4 |
| Dementia | 290 |
| Paralysis | 342, 344.1 |
| Diabetes without chronic complication | 250.0, 250.1, 250.2, 250.3, 250.7 |
| Diabetes with chronic complication | 250.4, 250.5, 250.6 |
| Mild liver disease | 571.2, 571.4, 571.5, 571.6 |
| Moderate-severe liver disease | 456.0, 456.1, 456.2, 572.2, 572.3, 572.4, 572.8 |
| Peptic ulcers | 531-534 |
| Rheumatoid arthritis | 714 |
| Malignancy | 140-149, 150-159, 180-189, 170, 171, 172, 174, 175, 176, 179, 160-165, 190-195, 200-208 |
| Metastatic solid tumour | 196-199 |
| **Others** |  |
| Stroke or systemic embolism | 433.01, 433.11, 433.21, 433.31, 433.81, 433.91, 434, 436, 437.0, 437.1, 444, 445 |
| Respiratory infections | 460-466, 480-488 |
| Viral infections | 053, 054, 058, 088.81, 042, 483.0 |
| Asthma | 493 |
| Congestive Heart Failure | 398.91, 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.13, 404.91, 404.93, 428 |
| Systemic lupus erythematosus | 710.0 |
| Spondylarthritis | 720.0 |
| Psoriasis | 696 |
| Multiple sclerosis | 340 |
| Inflammatory bowel disease | 555, 556 |

**Supplementary Table 2 Drug Name and British National Formulary (BNF) code used to identify prescription**

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| Description | Drug Name | BNF |
| Antibiotics for tuberculosis | isoniazid, rifampin, ethambutol, pyrazinamide, streptomycin | - |
| Statin | vastatin, lipitor, crestor, zocor, lescol, caduet |  |
| Angiotensin-converting enzyme | benazepril, captopril, enalapril, fosinopril, lisinopril, perindopril, quinapril, ramipril, trandolapril | - |
| Angiotensin Receptor Blockers | candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, valsartan | - |
| Digoxin | digoxin | - |
| Anticoagulant | - | 2.8.2 |
| Antiplatelet medications | - | 2.9 |
| Beta blocker | - | 2.4 |
| Calcium channel blockers | - | 2.6.2 |
| Antiviral drugs | - | 5.3 |
| Antibiotic drugs | - | 5.1 |
| Corticosteroids | - | 6.3 |
| Immunosuppressants | - | 8.2 |
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