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|  | Statistical Analysis Plan |
| Date | 13/05/2022 |
| Authors | 1. Xue Li
2. Kuan Peng
3. Franco WT Cheng
4. David Chi Leung Lam
5. Ching Lung Cheung
6. Celine Sze Ling Chui
7. Francisco Tsz Tsun Lai
8. Eric Yuk Fai Wan
9. Carlos King Ho Wong
10. Tiantian Ma
11. Samson Hin Hei Yum
12. Esther Wai Yin Chan
13. Jian Dong Huang
14. Chak Sing Lau
15. Mary Sau Man Ip
16. Ian Chi Kei Wong
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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 case-control studyProtocol version: version 1Patients: Hospital Authority active usersIntervention: two-dose SARS-CoV-2 vaccinationControl: unvaccinated individualsOutcome: Incident TB diagnosed from inpatient settings  |

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| title page |
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| Principal investigator(s): | Ian CK Wong, Xue Li |
| Nominated main analyst(s): | Kuan Peng |
| Nominated second/third independent analyst(s): | Franco W.T. Cheng |
| Publicly available on CARE website? <https://www.hkcare.hku.hk/>  | Yes [x]  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: Case control study with hospital controls |
| 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 anonymized EHRs include information about demographics, death, diagnoses, drug prescription records, hospitalization, attendances, and laboratory tests16Periods covered: 01/01/2018 to 01/31/2022Department of Health (DH) vaccination records database: The database consists of date of vaccination, vaccination sequence, vaccine brand, and recipient’s baseline informationPeriods covered: 02/23/2021 to 01/31/2022The 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: * Exclude subjects with 2 different brands of vaccine
* Exclude subjects aged <18 years
* Exclude subjects hospitalized but only receive one dose vaccine
* Exclude subjects with metastatic cancer before Feb 23, 2021
* Exclude subjects with TB diagnosis history (any setting) and prescription history (any one of the two drugs isoniazid and rifampin) before Feb 23, 2021
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| 4. | STUDY POPULATION |
|  | Case: Newly diagnosed TB cases (ICD-9-CM: 010-018) in inpatient setting with primary ranking since 2021-02-23 verified by isoniazid + rifampin + pyrazinamide + [ethambutol or streptomycin)] within 14 days after admission.Control: age, sex, admission date (±1 day) matched with admitted patients without the diagnosis of TB  |
| 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: NA |
| 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 allow adequate time for T-cell response triggered trained immunity. |
| 9 | ANALYSIS |
| 9a | Main analysis: Conditional logistic regression investigates the adjusted odds ratio comparing vaccinated group and unvaccinated group |
| 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 |
| 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.
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| 11 | RELEVANT RESEARCH CHECKLIST |
|  | STrengthening the Reporting of OBservational studies in Epidemiology for Case-Control 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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