Study Protocol

CoviBoost: Effect of BNT162b2 mRNA COVID-19 Vaccine booster immunization on SARS-CoV-2 antibody levels and breakthrough infection among healthcare workers

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Study Design:

A prospective, unblinded non-randomized, parallel group study.

Background

The COVID-19 pandemic has dramatically impacted healthcare systems worldwide[1-3]. Although most infected individuals remain asymptomatic or develop a mild disease, a significant proportion of patients will deteriorate and develop severe manifestations, often requiring respiratory support, resulting in either prolonged hospitalizations, long-term sequelae or even death[4].

The advent of efficient vaccines has dramatically changed the COVID-19 pandemic landscape. The vaccines have shown remarkable efficacy in preventing asymptomatic infection, symptomatic mild and severe disease, and death[5-7]. Effectiveness of the Pfizer-BioNTech BNT162b2 vaccine was demonstrated among healthcare workers in Israel[8]. All vaccine types evoke both humoral and cellular responses of varying intensities[9]. Unfortunately, vaccine effectiveness is decreasing across the globe, albeit at different rates in different countries, with the strongest decline in vaccine effectiveness recorded in Israel (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1005517/Technical_Briefing_19.pdf; https://www.gov.il/BlobFolder/news/06072021-04/he/NEWS_Corona_vaccine-eficacy.pdf). Three elements have been suggested to underlie this recent trend. First, relaxation of containment measures worldwide has been naturally accompanied by a surge of new cases, resulting in increased risk of infection. Second, new SARS-Cov-2 strains are continuously emerging, some of which seem to escape immune protection[10]. Of particular concern is the so-called delta strain that has been found to spread with great efficiency in the population in most European countries, in the US and in Israel. However, recent data from the UK suggest that the original vaccines retain most of their effectiveness against the delta variant[11]. Third, data from Europe and Israel

demonstrates decreasing antibody levels over time (https://www.medrxiv.org/content/10.1101/2021.07.07.21259499v1.full.pdf; 12). Several studies have shown a clear and strong correlation (whether causal or not) between antibody titers and protection against COVID-19[13,14]. The fact that the Israeli population received full vaccination earlier than the British population may explain the discrepancy between the marked decreases in vaccine effectiveness against infection observed in Israel as compared with that recorded in the UK (https://www.gov.il/en/departments/news/05072021-03; 11).

Populations at risk for infection and/or severe disease include the elderly as well as individuals with various chronic diseases[15]. Recently, the ministry of health (MOH) recommended to administer third vaccine dose to individuals older than 60 years who received 2 doses of BNT162b2. Health care personnel are more likely to be in contact with at-risk individuals and therefore are more likely to contract SARS-CoV-2 infection as well as to transmit it to individuals at-risk[16-19]. Moreover, availability of healthcare workers is a fundamental requirement to maintain healthcare systems` capacity. Thus, protecting healthcare workers is of utmost medical importance. There is uncertainty regarding the effectiveness of a third, “booster” injection to immunocompetent individuals in preventing infection with SARS-CoV-2. Given the steadily increasing numbers of new cases in Israel as well as the reassuring safety data available so far for individuals who were administered a third dose of COVID-19 vaccine (https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3890865; 20,21), we aim to examine the effectiveness of the third “booster” dose in a well-defined group of healthcare workers, employed at the Tel Aviv Sourasky Medical Center.

Study Objectives:

Primary objective:

To assess the effect of a third dose of BNT162b2 on the incidence of symptomatic and asymptomatic SARS-CoV-2 infection among Tel Aviv Sourasky Medical Center

healthcare workers with a low titer of antibodies against SARS-CoV-2 S protein compared to healthcare workers who did not receive a third vaccine dose.

The reason for performing this study among healthcare workers is detailed above. In short, healthcare workers are more likely to be in contact with infected individuals. Thus, they are likely to benefit most from the third dose of vaccine. In addition, protecting healthcare workers from infection is also likely to benefit their patients as explained above.

Secondary objectives:

1. To assess the safety and side effect profile of a third dose of BNT162b2 in participants with a low baseline titer of antibodies against SARS-CoV-2 S protein.
2. To assess the effect of a third dose of BNT162b2 on humoral and cellular immunity in participants with a low baseline titer of antibodies against SARS-CoV-2 S protein compared to healthcare workers who did not receive a third vaccine dose.
3. To assess the effect of a third dose of BNT162b2 on the infection rate in household contacts of the study participants who contracted SARS-CoV-2 throughout the study period.
   The reason for assessing the effect of a third vaccine dose on infection rate of household contacts is to perform contact tracing and evaluate the risk of transmission among healthcare workers who received thirds vaccine dose compared to healthcare workers who did not.

Sex:

Females and males.

Number of participants:

Up to 7,000 participants will be included in the screening phase of the study, which includes baseline antibody levels.

Up to 1,500 participants will be included in the intervention group and will be administered a booster dose of BNT162b2.

Rational for the sample size:

Assuming that 7000 healthcare workers will be tested for serology (anti-spike IgG), and 1500 subjects will have low anti-spike antibodies and would agree to receive a third vaccine dose, if the efficacy of the third vaccine dose is 40% and the incidence of positive tests is 5%, a sample size of 3000 subjects will be required (1500 each arm).

Ages:

18 years and over

Inclusion Criteria:

1. Male and female adults, aged 18 years and over
2. Healthcare workers (employees, volunteers, or students) at the Tel Aviv Medical Center
3. Receipt of a primary series (2 doses) of BNT162b2 (Pfizer/BioNTech COVID19 vaccine), at least 4 weeks prior to screening.
4. Willingness to comply with study procedures, including periodic blood sampling and nasopharyngeal swabs for PCR analysis. Of note, willingness to receive a booster dose of BNT162b2 is not an inclusion criterion (see below).

Exclusion Criteria:

1. Previous severe adverse reaction to BNT162b2.
2. Known anaphylactic reaction to one or more component of BNT162b2.
3. Known type-1 allergy (including urticaria, angioedema, etc) to one or more component of BNT162b2.
4. Current pregnancy.
5. Previous or current infection with SARS-CoV-2, as documented by present or past positive PCR for SARS-CoV-2 RNA from nasal or pharyngeal specimen.
6. Healthcare workers who are under immunosuppressive medications for rheumatological diseases, malignancy or solid organ transplantation.

Special Populations:

The study will not include special populations such as pregnant women, persons who are not able to provide informed consent or those under 18 years of age.

Study Description:

1. Screening phase:

   Healthcare workers of the Tel Aviv Sourasky Medical Center will be offered through all organizational communication channels to participate to the CoviBoost study. Healthcare workers who meet inclusion criteria and do not meet exclusion criteria, after providing informed consent, will be included in the study cohort and will complete a questionnaire aimed at collecting baseline demographic and medical data.

2. Intervention phase:

Participants will undergo a blood test (a total of 10 ml) to determine the level of SARS-CoV-2 antibodies levels. A sample of 400 participants (of the entire study cohort) will undergo a blood test (additional 10 ml) for cellular immunity to SARS-

CoV-2. Cellular immunity will be ascertained as previously reported[22]. In brief, the evaluation of specific cellular immune response against the SARS-CoV-2 S-protein will be performed by stimulation donor cells with a pool of lyophilized peptides of the viral Spike glycoprotein (S), followed by staining for the surface activation marker CD154 (CD40L) and for the intracellular cytokines IFNγ and TNFα. For this purpose, frozen PBMCs will thawed, plated in a 96-well plate at a concentration of $1X10^6$ cells / 100μL, and stimulated for 6 hours with S-peptide pool (Miltenyi's PepTivator® SARS-CoV-2 Prot_S complete) and relevant controls, in the presence of Brefeldin A. Cells will then fixed permeabilized and stained for the surface markers CD3, CD4, CD8 and CD154, and intracellularly for intracellularly for IFNγ and TNFα. Stained samples will be acquired using BD FACSCanto II flow cytometer and analyzed using FlowJo software (V10.0, TreeStar). Responding cells will be identified by upregulation of CD154 and intracellular cytokine production.

Serologic testing for anti-spike protein (anti-S1) IgG will be performed using indirect chemiluminescent microparticle immunoassay on the ADVIA Centaur XP system (SIEMENS). Low antibody levels will be considered below the 50% percentile of the IgG levels of the study population.). Participants with low or undetectable levels of anti-spike antibodies against SARS-CoV-2 will be offered a third dose of BNT162b2 (0.3 ml intra-muscular injection).

Participants with low or undetectable levels of anti-spike antibodies against SARS-CoV-2 who refuse to receive the vaccine will serve as a control group. In addition, participants with detectable levels of anti-spike antibodies will continue to be monitored during the study period.

3. Monitoring phase:

   Participants included in the study will be categorized into three groups: those who received a 3rd dose of BNT162b2 as well as those who did not, whether due to high baseline antibody levels or due to refusal to receive a 3rd dose. All

participants will undergo repeat blood tests for antibody and cell mediated immunity at 1,3,6 months and will also be requested to complete a nasal swab for SARS-CoV-2 PCR every 14 days, for a period of at least 3 months and up to 12 months. In addition to periodic PCR testing, nasal and pharyngeal swabs will be obtained in response to symptoms onset or as part of an epidemiological investigation.

The hospital's virological laboratory performs RT-PCR testing using several assays: 1) the Seegene Allplex™ 2019-nCoV assay, targeting the E, N and RdRP genes; 2) the cobas® SARS-CoV-2 assay, targeting the E and the ORF genes; 3) the Xpert® Xpress SARS-CoV-2, targeting the E and the N genes; 4) the Simplexa™ COVID-19 Direct assay, targeting the S and the ORF genes. Cycle threshold value of each gene will be documented.

Participants who received a third dose will be requested to complete a questionnaire regarding side effects and adverse events 7 and 30 days after receipt of the vaccine.

Owing to the pandemic dynamics, it is possible that the government decides to vaccinate all healthcare workers in addition to the current recommendation to vaccinate persons older than 60 years. In such scenario, participants in the control group who choose to receive the third vaccine dose outside the study protocol will be asked to be followed during the study period. This is also valid for participants older than 60 years old who are currently being vaccinated based on the government decision.

In addition, participants who decide to be in the control group when signing the informed consent and change their minds will be allowed to receive the third vaccine dose and asked to continue the study follow-up.

Participants may choose to receive the third vaccine dose in the community and not in the hospital (the study center) will be asked to do so after being tested for

serology and present the vaccination confirmation to the primary investigator . These participants will continue follow-up according to the study protocol, including reporting for adverse reactions if occur, having nasal and pharyngeal swabs for PCR based on study indications, and tested for serology.

Data Collected:

1. Demographics including age, sex, employment sector and department,
2. Past medical history including vaccination dates, co-morbid conditions and medications
3. Serology and cellular immunity assay results at baseline and at known intervals
4. Dates and results of periodic SARS-CoV-2 testing as well as indications for each test (due to symptoms, known exposure to a SARS-CoV-2 positive individual, return from an endemic country or in response to any other ministry of health's policy, or as part of routine study requirements).
5. For participants who are administered a third dose of BNT162b2, information regarding self-reported side effects and/or adverse events.
6. For SARS-CoV-2 positive cases, results of epidemiological investigations including known source of infection, presence and details of symptoms, information on household contacts who contracted SARS-CoV-2 within 14 days after the date of infection diagnosis, and laboratory data such as cycle threshold and target genes examined.

Serious adverse events will be reported to the ministry of health per MOH requirements.

Each participant will be assigned a unique identifying number and that number will be used throughout all data collection and analysis processes.

The mapping of study identifiers and participant details will be stored by the primary investigator in a locked cabinet or a password-protected hospital-issued computer. The mapping list will be deleted or physically destroyed after completion of data analysis, and a report of said deletion will be included in the study summary. This file will not be

sent via unencrypted email nor will it be removed from hospital premises without written consent by the hospital's R&D division.

Only the primary and secondary investigators, who are hospital employees with permission to access medical records, will have access to the de-identified data.

Study Duration:

12 months.

The study site will be monitored to assess compliance with the trial protocol and general principles of Good Clinical Practice by a person who is not a part of the study team.

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