PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in the Bruneian population: Protocol for a national longitudinal study
AUTHORS	Ghani, Hazim; Ahmad, Liyana; Sharif, Hanisah; Wong, Justin; Bagol, Saifuddien; Alikhan, Mohammad Fathi; Taib, Surita; Tan, Chee Wah; Zhu, Feng; Ong, Xin Mei; Shim, Chin Yee; Wang, Yan; Chan, Si Yee; Wei, Yuan; Idris, Fazean; Naing, Lin; Wang, Lin-Fa; Cunningham, Anne

VERSION 1 – REVIEW

REVIEWER	Tuğba Y. Yalçın Baskent Universitesi
REVIEW RETURNED	25-Aug-2022

GENERAL COMMENTS	Dear authors, First of all, I would like to say that your study excites me. However, I have some suggestions Could pregnancy or being pregnant also be an exclusion criteria? The course of SARS-CoV-2 variants in symptomatic disease can be very different. Is being infected with different variants a limitation? I suggest limitations will be expanded. Many studies have also shown that the severity of the disease affects the antibody response. It is important to consider the severity of the disease in breakthrough infections. There are many confounding factors in the assessment of vaccine response (such as body mass index, diet, sleep status, smoking, alcohol use, etc.) In the interim evaluations, participants should be thoroughly questioned. Symptoms of disease, hospital admissions, newly emerging comorbidities (such as diabetes, hypertension, use of immunosuppressive drugs..) I think other vaccine applications should be questioned (tetanus, rabies, hepatitis A or B..) Good luck with your work

REVIEWER	Daniele Focosi Pisa University Hospital, North Western Blood Bank
REVIEW RETURNED	17-Sep-2022

GENERAL COMMENTS	The paper is well written, but the protocol does not include any novelty at all: usage of surrogate viral nuetralization assays has already been implemented in large population screenings. Also, being the test not based on BA.4/5, it is unlikely to inform public health decision. Pease change BNT1626b into BNT162b2 throughout the manuscript.

REVIEWER	Jessica Tuan Yale University School of Medicine Section of Digestive Diseases
REVIEW RETURNED	15-Oct-2022

GENERAL COMMENTS	The study brings awareness and light to SARS-CoV-2 immunogenicity post-vaccination in an underreported population in Southeast Asia. It is an interesting study which utilizes advanced electronic health record technology. In the opening line, rather than 'predictive,' would use the word 'correlative,' given neutralizing antibodies do not necessarily predict immunoprotection. Of note, the booster series options are different from the primary series which makes a one-to-one comparison challenging, which would include in study limitations. Study limitations should also highlight limitation of not having pre-vaccination serologies. Please clarify by BNT1626b (line 77) if you mean BNT162b2. Details of the cPassTM surrogate virus neutralization test should be outlined in the Methods section (e.g. ELISA?). Please describe if the cutoff of 30% has been validated for "positive" or "negative." In the Methods section, statistical analysis that will be performed should be clarified. Outcomes should be more clearly defined, are there Geometric mean titers that will be evaluated. Change "High level" to "High levels" (line 92). Please clarify if SARS-CoV-2 nucleocapsid antibody testing was performed prior to enrollment to exclude those with prior COVID-19 history from the study.

VERSION 1 – AUTHOR RESPONSE

Comments from Reviewer #1:

Dear authors,

First of all, I would like to say that your study excites me. However, I have some suggestions

Major comment 1:

Could pregnancy or being pregnant also be an exclusion criteria?

Response:

Thank you for your comment. We agree with Reviewer #1 that pregnancy could influence NAb levels and potentially have been an exclusion criterion. However, the main aim of the study is to investigate

the levels of NAb following vaccination over time. Therefore, we identified prior exposure to the SARS-CoV-2 virus as the main factors that could affect the NAb levels (duration from the last infection). This included traveling and a prior history of COVID-19 infection as the only exclusion criteria for Phase 1.

Major comment 2:

The course of SARS-CoV-2 variants in symptomatic disease can be very different. Is being infected with different variants a limitation? I suggest limitations will be expanded.

Response:

Thank you for your comment. We agree with Reviewer #1 that different SARS-CoV-2 variants may present with different courses of infection. However, it was not feasible to identify the specific SARS-CoV-2 variant(s) in individual participants with breakthrough infection. The following sentence has been added to the study limitation:

"Hence, there will be limited information on the specific SARS-CoV-2 variants in the breakthrough infection cohort." (marked manuscript, pages 5-6, lines 75-77)

Major comment 3:

Many studies have also shown that the severity of the disease affects the antibody response. It is important to consider the severity of the disease in breakthrough infections.

Response:

Thank you for your comment. Indeed, studies have shown that individuals with breakthrough infections present with different severity and can affect NAb levels. In our study cohort, only mild cases were reported. These cases do not require emergency medical attention or life support. Nonetheless, any severe cases will be recorded and associated with NAb levels. The following sentence has been added to clarify this:

"The symptoms and severity of breakthrough infections in these participants may be analyzed and compared to those without breakthrough infections." (marked manuscript, page 16, lines 254-255)

Major comment 4:

There are many confounding factors in the assessment of vaccine response (such as body mass index, diet, sleep status, smoking, alcohol use, etc.) In the interim evaluations, participants should be thoroughly questioned. Symptoms of disease, hospital admissions, newly emerging comorbidities (such as diabetes, hypertension, use of immunosuppressive drugs..)

Response:

Thank you for your comment. Information on co-morbidities is recorded as part of the study variables, including diabetes mellitus, chronic kidney disease, hypertension, ischemic heart disease and cancer, in addition to having a history of or ongoing immunosuppressive drug treatment. A change in the status of any of the co-morbidities information or drug history will be checked during each appointment, and any changes will be recorded accordingly. The following sentence has been added to clarify this:

“Socio-demographic data including age, gender, ethnicity, co-morbidities (i.e., diabetes mellitus, chronic kidney disease, hypertension, ischemic heart disease and cancer) and immunosuppressive drug use will be enquired and recorded in a secured online platform” (marked manuscript, page 16, lines 248-251)

Major comment 5:

I think other vaccine applications should be questioned (tetanus, rabies, hepatitis A or B..)

Response:

Thank you for your comment. It is true that there may be potential cross-reactivity between different vaccines that could lead to an immune response against SARS-CoV-2. However, this does not align with the main aim and focus of the study, which sought to investigate the effects of vaccination on NAb levels using an inactivated virus, viral vector and mRNA vaccine platforms.

Comments from Reviewer #2:

Major comment 1:

The paper is well written, but the protocol does not include any novelty at all: usage of surrogate viral nuetralization assays has already been implemented in large population screenings.

Response:

Thank you for your comment. Indeed, several studies have utilized surrogate viral neutralization tests in a population study. However, there is very limited population data in a Southeast Asian cohort apart from Singapore (others include China and Monaco for this particular surrogate virus neutralization test). Thus, this study will contribute valuable comparisons to previously published data. Furthermore, it is worth noting that this study aims to recruit a high number of participants and is mentioned as a Strength of the study (marked manuscript, page 5, lines 57-60).

Major comment 2:

Also, being the test not based on BA.4/5, it is unlikely to inform public health decision.

Response:

Thank you for your comment. Indeed, this assay detects neutralizing antibodies against the ancestral SARS-CoV-2 strain. Nevertheless, the immune response can exhibit cross-reactivity between the different SARS-CoV-2 variants. Although neutralizing antibodies do not lead to prevention of the disease, it can provide protection against severe illness or long COVID symptoms. Furthermore, this study can provide information regarding the population-specific immunogenic response elicited following the administration of different vaccine platforms, which would be helpful from a public health perspective. Nonetheless, this has been stated as a Limitation of the study (marked manuscript, page 5, lines 65-69).

Major comment 3:

Please change BNT1626b into BNT162b2 throughout the manuscript.

Response:

Thank you for your comment. We apologize for the inconvenience caused. This has now been rectified throughout the manuscript.

Comments from Reviewer #3:

The study brings awareness and light to SARS-CoV-2 immunogenicity post-vaccination in an underreported population in Southeast Asia. It is an interesting study which utilizes advanced electronic health record technology.

Major comment 1:

In the opening line, rather than 'predictive,' would use the word 'correlative,' given neutralizing antibodies do not necessarily predict immunoprotection.

Response:

Thank you for your comment. This has now been amended (marked manuscript, page 3, line 26).

Major comment 2:

Of note, the booster series options are different from the primary series which makes a one-to-one comparison challenging, which would include in study limitations. Study limitations should also highlight limitation of not having pre-vaccination serologies.

Response:

Thank you for your comment. We agree with Reviewer #3 that pre-vaccination sampling would provide valuable insight into the changes in NAb levels following administration of the vaccines. Furthermore, this would provide information on potential exposure to SARS-CoV-2 virus prior to the

study. Unfortunately, this was not feasible as there was a national lockdown during the commencement of the study. Consequently, it was challenging to recruit unvaccinated individuals for this study.

We also agree that ideally homologous series of vaccinations (same vaccine administered as the primary and booster doses) would provide a more relevant comparison between the different vaccine platforms. However, the viral vector (AZD1222) vaccine was not offered as a booster in the National Vaccination Programme in Brunei; mainly mRNA vaccines (mRNA-1273 and BNT162b2) and only a small proportion of whole inactivated virus (BBIBP-CorV) vaccine are offered as booster vaccine platforms. Likewise, BNT162b2 was also only offered as a booster dose for the adult population. Thus, we were limited with the combination of the primary and booster doses as stated in the manuscript.

The following sentences have been added as a Limitation of the study:

“Pre-vaccination blood serum sampling was not performed to indicate potential neutralizing antibodies present in participants. Moreover, comparisons of homologous vaccinations using AZD1222 and BNT162b2 were limited due to the difference in vaccine platforms distributed in the primary series and booster vaccinations.” (marked manuscript, page 5, lines 70-73)

Major comment 3:

Please clarify by BNT1626b (line 77) if you mean BNT162b2.

Response:

Thank you for your comment. We apologize for the inconvenience caused. This has now been rectified throughout the manuscript.

Major comment 4:

Details of the cPassTM surrogate virus neutralization test should be outlined in the Methods section (e.g. ELISA?). Please describe if the cutoff of 30% has been validated for "positive" or "negative."

Response:

Thank you for your comment. The nature of the cPass kit has been briefly mentioned in the Introduction section as an ELISA-based assay (marked manuscript, pages 7-8, lines 101-105). This assay has previously been validated in a study by Tan *et al.* (reference number 18) which uses the 30% inhibition as a cutoff point between negative and positive NAb level readings. Further elaboration on the process of the cPass kit as an ELISA-based assay is presented in the Methods section (marked manuscript, page 17, lines 263-278).

Major comment 5:

In the Methods section, statistical analysis that will be performed should be clarified.

Response:

Thank you for your comment. Apologies for the inconvenience caused. We have now specified the statistical tests that will be used in the study to include “using the Mann-Whitney U and Kruskal-Wallis tests with 95% confidence interval (CI)” (marked manuscript, page 20, lines 311-313).

Major comment 6:

Outcomes should be more clearly defined, are there Geometric mean titers that will be evaluated.

Response:

Thank you for your comment. Geometric mean titers (GMT) will be evaluated. We have now specified the conversion of percentage inhibition to antibody titers (using a WHO-approved International Standard tool) (Zhu et al, 2022; reference number 22). GMT will be evaluated per group and will be used to compare the different vaccine platforms and subgroups during statistical analyses (marked manuscript, page 20, line 311).

Major comment 7:

Change "High level" to "High levels" (line 92).

Response:

Thank you for your comment. This has now been amended as suggested.

Major comment 8:

Please clarify if SARS-CoV-2 nucleocapsid antibody testing was performed prior to enrollment to exclude those with prior COVID-19 history from the study.

Response:

Thank you for your comment. Unfortunately, there was no pre-testing performed, including the stated SARS-CoV-2 nucleocapsid antibody, to confirm prior COVID-19 history or lack thereof. Recruitment of participants who have not been exposed to the virus was only based on the national electronic health record databases, and participants were further enquired during blood sampling if they had been previously infected. The following sentence has been added as a Limitation of the study:

“Pre-vaccination blood serum sampling was not performed to indicate potential neutralizing antibodies present in participants.” (marked manuscript, page 5, lines 70-71)