BRIEF REPORT

Slow waning of antibodies following a third dose of BNT162b2 in adults who had previously received two doses of inactivated vaccine

Benjamin J. Cowling[1,2], Samuel M. S. Cheng[1], Mario Martín-Sánchez[1], Niki Y. M. Au[1], Karl C. K. Chan[1], John K. C. Li[1], Lison W. C. Fung[1], Leo L. H. Luk[1], Leo C. H. Tsang[1], Dennis K. M. Ip[1], Leo L. M. Poon[1,3,4], Gabriel M. Leung[1,2], J. S. Malik Peiris[†1,3,4], Nancy H. L. Leung[†1,2]

[†]Joint senior authors with equal contribution

Affiliations:

1. WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China.
2. Laboratory of Data Discovery for Health; Hong Kong Science and Technology Park, New Territories, Hong Kong Special Administrative Region, China.
3. HKU-Pasteur Research Pole, School of Public Health, Li Ka Shing Faculty of Medicine, Pokfulam, The University of Hong Kong; Hong Kong Special Administrative Region, China.
4. Centre for Immunology and Infection; Hong Kong Science and Technology Park, New Territories, Hong Kong Special Administrative Region, China.

Corresponding author:

Benjamin Cowling, WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, LKS Faculty of Medicine, The University of Hong Kong; Hong Kong Special Administrative Region, China. bcowling@hku.hk

Alternate corresponding author in the event that the corresponding author is unavailable: Nancy Leung, leungnan@hku.hk

Running head: Robust immunity after BNT162b2 booster

Word count (abstract): 79

Word count (main text): 1,997

ABSTRACT

We administered BNT162b2 as a third dose to 314 adults aged ≥30 years who had previously received two doses of inactivated vaccination. We collected blood samples before the third dose and again after one month and six months, and found robust antibody responses to the ancestral strain at six months after receipt of BNT162b2. Antibody responses to Omicron BA.2 by live virus neutralization were weaker after the third dose and had declined to a low level by six months.

Key words: COVID-19, BNT162b2, CoronaVac, immunogenicity, antibody

INTRODUCTION

Third doses of COVID-19 vaccination provide an important boost to immunity, reducing the risk of symptomatic infection and the risk of severe disease. Third doses have been particularly important for improving protection against variants. However, waning clinical protection particularly against Omicron was noted after receipt of third doses, with fourth doses then providing additional protection [1]. In recipients of two initial doses of inactivated COVID-19 vaccines, we and others have shown that a third "booster" dose of BNT162b2 (BioNTech/Pfizer/Fosun Pharma) confers a very strong antibody response both against the ancestral strain and the Omicron variant [2-4]. Here, we explore the persistence of antibody titers up to 6 months after a third dose of BNT162b2 in this regimen. Prior to the start of our study and during the period when we administered BNT162b2 there were very few COVID-19 infections in Hong Kong [5], while the subsequent follow-up period included a large local epidemic of Omicron BA.2 in early 2022 [6].

METHODS

Study design

We conducted an open-label single-arm trial to measure the antibody responses to a third dose of BNT162b2 in adults ≥30 years of age who previously received two doses of an inactivated COVID-19 vaccine with the second inactivated vaccine dose at least 90 days prior to enrolment [2]. Participants were not eligible if they had a history of laboratory-confirmed COVID-19 infection, if they met a contraindication for BNT162b2, were receiving immuno-modulatory medications, or were females who were pregnant or intending to become pregnant in the upcoming 3 months [2].

Each participant provided a serum sample at Day 0 prior to receipt of BNT162b2, and then further serum samples on Day 28 (±7 days) and Day 182 (±30 days), with a final sample planned on Day 365. We collected information at baseline on demographics, health status including vaccinations received, and self-reported COVID-19 infection history. We updated this information at the Day 182 visit including information on any infections that had occurred between Day 28 and Day 182.

Ethical approval

All participants provided written informed consent. The study was approved by the Institutional Review Board of the University of Hong Kong. The study is registered on Clinicaltrials.gov (NCT05057182).

Laboratory methods

We used a SARS-CoV-2 Spike RBD IgG enzyme-linked immunosorbent assay (ELISA) for the ancestral strain as previously described [7]. 96-well ELISA plates (Nunc MaxiSorp, Thermo Fisher Scientific) were coated overnight with 100ng/well of the purified recombinant RBD protein in PBS buffer. The plates were then blocked by 100µl of Chonblock blocking buffer (Chondrex Inc, Redmond, US) per well and incubated at room temperature for 2 hours. Each serum sample was tested at a dilution of 1:100 in Chonblock blocking buffer in duplicate. They were added and were incubated for 2 hours at 37°C. After extensive washing with PBS containing 0.1% Tween 20, horseradish peroxidase-conjugated goat anti-human IgG (1:5000, GE Healthcare) was added and incubated for 1 hour at 37°C. The ELISA plates were then washed

again with PBS containing 0.1% Tween 20. Subsequently, 100µL of HRP substrate (Ncm TMB One; New Cell and Molecular Biotech Co. Ltd, Suzhou, China) was added into each well. After 15 minutes the reaction was stopped by adding 50µL of 2 M H_2SO_4 solution and analyzed on a microplate reader at 450nm wavelength. Optical density above 0.5 was considered positive.

SARS-CoV-2 surrogate virus neutralization test (sVNT) kits (Cat. No.: L00847-A) were ordered from GenScript, Inc., NJ, USA. The tests were performed according to the manufacturer's standard protocol. 10X dilutions were performed for samples, positive and negative controls. They were then mixed with an equal volume of horseradish peroxidase-conjugated SARS-CoV-2 spike RBD (6ng). The mixture was incubated at 37°C for 30 min. After incubation, 100µl of the mixture was added to corresponding wells of the capture plate coated with ACE-2 receptor. The plate was sealed and incubated at 37°C for 30 min followed by removing mixtures and washing with 1X wash solution four times, emptying residual liquid by tapping dry. 100µl of TMB solution was added to each well, the plate was wrapped with aluminium foil and incubated in the dark at room temperature for 15 minutes. The reaction was quenched by adding 50µl of stop solution. The absorbance was read at 450nm (OD_{450}) in an ELISA microplate reader. Percentage inhibition was calculated by (1-OD_{450} value of sample/OD_{450} value of negative control) times 100%.

The Plaque Reduction Neutralization Test (PRNT) was performed in duplicate using 24-well tissue culture plates (TPP Techno Plastic Products AG, Trasadingen, Switzerland) in a biosafety level 3 facility using Vero E6 TMRESS2 cells [8] for the ancestral strain and Omicron BA.2 as previously described [9]. All sera were heat-inactivated at 56°C for 30 min before testing. Serial

two-fold dilutions from 1:10 to 1:320 of each serum sample were incubated with 30-40 plaque-forming units of virus for one hour at 37°C and the mixture was added onto pre-formed cell monolayers. The culture plate was incubated for 1 hour at 37°C in a 5% CO_2 incubator. The virus-antibody inoculum was then discarded, and the cell monolayer was overlaid with 1% agarose in cell culture medium. The plates were fixed and stained after 3 days incubation. Antibody titres were defined as the reciprocal of the highest serum dilution that resulted in ≥50% reduction in the number of virus plaques ($PRNT_{50}$). Virus back titrations, positive and negative control sera were included in every experiment.

Statistical analysis

We analyzed data on antibody titers measured by the assays listed above at Day 0, Day 28 and Day 182. We determined whether participants reported a laboratory-confirmed infection between Day 28 and Day 182, or had chosen to receive a fourth dose of a COVID-19 vaccine during the same period, and classified accordingly for analysis. We estimated group means for the ELISA and sVNT percentages, and geometric mean titers for the live virus neutralization titers, and also compared by age and sex. We estimated the rate of waning in neutralizing antibody titers for those who were not infected and did not receive a fourth dose assuming an exponential rate of decline. Statistical analyses were conducted using R version 3.6.2.

RESULTS

We administered BNT162b2 as a third dose of COVID-19 vaccination to 314 participants between 18 October and 28 December 2021. We collected Day 28 samples from 312 (99%) and Day 182 samples from 284 (90%) participants between 20 April and 1 June 2022. Among these

284 participants, 279 (98%) had received two initial doses of CoronaVac and the remainder received two doses of BIBP. The median delay between the second dose of inactivated vaccination and the third dose of BNT162b2 was 205 days (range 94, 267 days). The median age was 53 years, 29% of participants were ≥60 years of age, 38% were female, and 93 (32%) had a chronic medical condition.

Among the 284 participants, 42 (15%) reported a COVID-19 infection between receipt of the third dose and collection of the Day 182 sample, and 21 (7.0%) reported receipt of a fourth dose prior to collection of the Day 182 sample including one participant who was infected as well as then receiving a fourth dose. Among the 42 infections, 30 (71%) occurred in March 2022, with nine in February and three in April. The median delay from infection to collection of the Day 182 sample was 55 days (range 10, 85 days). The median delay from the fourth dose to collection of the Day 182 sample was 19 days (range 7, 26) and 19 participants received BNT162b2 as a fourth dose while two received CoronaVac.

The third dose of BNT162b2 led to substantial increases in ELISA (Figure 1A) and surrogate virus neutralization levels (Figure 1B) at Day 28, which waned somewhat by Day 182 but still remained substantially higher than the levels at Day 0. ELISA values at Day 182 were statistically significantly higher in the small number of participants who were infected ($p<0.001$, t-test) or received a fourth dose ($p=0.005$, t-test) prior to Day 182. The sVNT responses were very high at Day 182 in all groups, but also statistically significantly higher in the small number of participants who were infected ($p=0.002$, t-test) or received a fourth dose ($p=0.032$, t-test)

prior to Day 182. There were no statistically significant differences in ELISA or sVNT levels by age or sex at Day 182.

We measured $PRNT_{50}$ titers against the ancestral strain (Figure 1C) and Omicron BA.2 (Figure 1D) in a subset of 39 participants. In the statistical comparisons within this subset that follow we excluded (because of the small sample sizes) from Day 182 calculations the three infected participants and the two who received a fourth dose, although they are included in Figure 1 for completeness. At Day 28 and Day 182 the geometric mean $PRNT_{50}$ titers against the ancestral strain were 338 and 112, respectively. The corresponding geometric mean $PRNT_{50}$ titers against BA.2 were 55 and 14, respectively. There were no statistically significant differences by age or sex in $PRNT_{50}$ titers against the ancestral strain or BA.2 at Day 182. Assuming an exponential rate of waning from Day 28 to Day 182, we estimated that antibody titers would drop by half in 96 days for the ancestral strain and 79 days for BA.2.

DISCUSSION

We show durable antibody responses to the ancestral strain six months after the third dose of BNT162b2 (Figure 1), consistent with other studies that show a strong and sustained antibody response to a third dose of BNT162b2 after two doses of BNT162b2 [10] or after two doses of inactivated vaccination [11]. Antibody titers measured by sVNT against the ancestral strain were higher at 97% six months after the third dose of BNT162b2 (and two earlier doses of CoronaVac) than six months after either two doses of BNT162b2 or two doses of CoronaVac, when sVNT inhibition had fallen to 80% and 20% respectively in another study [12]. However, neutralizing titers to Omicron BA.2 only reached a moderate geometric mean titer of 55 after the

third dose, above a threshold thought to provide some degree of protection against infection in this assay [9] but titers had fallen below a geometric mean of 14 within six months (Figure 1D), potentially below the protective threshold. One interesting observation in our study is the appearance of a more rapid decline in neutralizing titers (Figures 1C and 1D) compared to binding antibody titers (Figure 1A) by Day 182.

There is some evidence from observational studies that third doses can protect against symptomatic Omicron BA.2 infection [13]. In studies of the effectiveness of two and three doses of COVID-19 vaccines in Hong Kong we found evidence suggestive of a moderate level of protection against mild infection [14]. From a small number of participants we observed that natural infection or a fourth dose of vaccination generated similar antibody levels against the ancestral virus, but infection may have generated higher antibody level against Omicron BA.2 than vaccination suggesting a potential advantage in the breadth of antibody response from hybrid immunity [15]. Further studies are needed to confirm this finding and to determine the optimal timing of fourth doses under different types of prior immunity.

Our study had a number of limitations. A large wave of Omicron BA.2 occurred in Hong Kong in February-April 2022 with more than a million confirmed cases (14% of the population) and 9000 deaths [6]. Many infections likely were undocumented. While 15% of our cohort reported an infection, including some infections that may not have been documented in the official case count, some other participants may have had an unrecognized infection, biasing upwards the antibody titers at Day 182. In addition, we did not measure cell-mediated immune parameters which may also contribute to protection.

In conclusion, a third dose of BNT162b2 provided a strong and durable immune response in adults who had previously received two doses of inactivated COVID-19 vaccine. Further research is needed on the value of immunogenicity data, including cellular immunity measures as well as non-neutralizing antibody levels, to predict the clinical effectiveness of booster doses against symptomatic disease and severe disease with Omicron subvariants.

ACKNOWLEDGEMENTS

We gratefully acknowledge colleagues including Zacary Chai, Sara Chaothai, Kelvin Kwan, Yvonne Ng, Teresa So and Eileen Yu for technical support in preparing and conducting this study; Anson Ho for setting up the database; Julie Au and Lilly Wang for administrative support; Hetti Cheung, Victoria Wong, Bobo Yeung at HKU Health System; Cindy Man and other colleagues at the HKU Community Vaccination Centres at Gleneagles Hospital; and all the study participants for facilitating the study.

FUNDING

This project was supported by the Theme-based Research Scheme T11-705/21-N of the Research Grants Council of the Hong Kong Special Administrative Region, China (BJC). BJC is supported by a RGC Senior Research Fellow Scheme grant (HKU SRFS2021-7S03) from the Research Grants Council of the Hong Kong Special Administrative Region, China. The funding bodies had no role in the design of the study, the collection, analysis, and interpretation of data, or writing of the manuscript.

AUTHOR CONTRIBUTIONS

All authors meet the ICMJE criteria for authorship. Each author's contributions to the paper are listed below according to the CRediT model:

Conceptualization: BJC, GML, NHLL

Methodology: BJC, SMSC, NHLL

Formal analysis: BJC, MM-S

Investigation: NYMA, KCKC, JKCL, LWCF, LLHL

Funding acquisition: BJC

Project administration: BJC, SMSC, JSMP, NHLL

Supervision: BJC, SMSC, DKMI, LLMP, GML, JSMP, NHLL

Writing – original draft: BJC

Writing – review & editing: BJC, SMSC, MM-S, NYMA, KCKC, JKCL, LWCF, LLHL, DKMI, LLMP, GML, JSMP, NHLL.

COMPETING INTERESTS

BJC consults for AstraZeneca, Fosun Pharma, GlaxoSmithKline, Moderna, Pfizer, Roche and Sanofi Pasteur. BJC has received research funding from Fosun Pharma. The authors report no other potential conflicts of interest.

REFERENCES

1. Bar-On YM, Goldberg Y, Mandel M, et al. Protection by a Fourth Dose of BNT162b2 against Omicron in Israel. N Engl J Med **2022**; 386:1712-20.

2. Leung NHL, Cheng SMS, Martin-Sanchez M, et al. Immunogenicity of a third dose of BNT162b2 to ancestral SARS-CoV-2 & Omicron variant in adults who received two doses of inactivated vaccine. Clin Infect Dis **2022**.

3. Campos GRF, Almeida NBF, Filgueiras PS, et al. Booster dose of BNT162b2 after two doses of CoronaVac improves neutralization of SARS-CoV-2 Omicron variant. Commun Med (Lond) **2022**; 2:76.

4. Leung NHL, Cheng SMS, Cohen CA, et al. Homologous and heterologous boosting with CoronaVac and BNT162b2: a randomized trial (the Cobovax study) medRxiv. medRxiv **2022**:doi: https://doi.org/10.1101/2022.08.25.22279158.

5. Chen LL, Abdullah SMU, Chan WM, et al. Contribution of low population immunity to the severe Omicron BA.2 outbreak in Hong Kong. Nat Commun **2022**; 13:3618.

6. Mefsin YM, Chen D, Bond HS, et al. Epidemiology of Infections with SARS-CoV-2 Omicron BA.2 Variant, Hong Kong, January-March 2022. Emerg Infect Dis **2022**; 28:1856-8.

7. Perera RAPM, Mok CKP, Tsang OTY, et al. Serological assays for SARS-CoV-2. Eurosurveillance **2020**; 25:pii=2000421.

8. Matsuyama S, Nao N, Shirato K, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci U S A **2020**; 117:7001-3.

9. Lau EH, Hui DS, Tsang OT, et al. Long-term persistence of SARS-CoV-2 neutralizing antibody responses after infection and estimates of the duration of protection. EClinicalMedicine **2021**; 41:101174.

10. Eliakim-Raz N, Stemmer A, Leibovici-Weisman Y, et al. Three-month follow-up of durability of response to the third dose of the SARS-CoV-2 BNT162b2 vaccine in adults aged 60 years and older: a prospective cohort study. BMJ Open **2022**; 12:e061584.

11. Silva ARD, Jr., Villas-Boas LS, Paula AV, et al. Neutralizing antibodies against the SARS-CoV-2 Omicron variant following two CoronaVac vaccinations and a Pfizer/BioNTech mRNA vaccine booster. Rev Inst Med Trop Sao Paulo **2022**; 64:e43.

12. Cowling BJ, Wong IOL, Shiu EYC, et al. Strength and durability of antibody responses to BNT162b2 and CoronaVac. Vaccine **2022**; 40:4312-7.

13. Chemaitelly H, Ayoub HH, AlMukdad S, et al. Duration of mRNA vaccine protection against SARS-CoV-2 Omicron BA.1 and BA.2 subvariants in Qatar. Nat Commun **2022**; 13:3082.

14. McMenamin ME, Nealon J, Lin Y, et al. Vaccine effectiveness of two and three doses of BNT162b2 and CoronaVac against COVID-19 in Hong Kong. Lancet Infect Dis **2022**.

15. Epsi NJ, Richard SA, Lindholm DA, et al. Understanding 'hybrid immunity': comparison and predictors of humoral immune responses to SARS-CoV-2 infection and COVID-19 vaccines. Clin Infect Dis **2022**.

FIGURE LEGEND

Figure 1. Antibody titers measured prior to receipt of a third dose of BNT162b2 (Day 0), and at Day 28 and Day 182 following that dose, using four assays. Samples collected at Day 182 were stratified by whether the participant had been infected or received a fourth dose between Day 28 and Day 182. Panel A: antibody titers measured by an ELISA assay for serum IgG against the receptor binding domain (RBD) of the spike protein of the ancestral strain, with X indicating the median level. Panel B: Responses to a surrogate virus neutralization test (sVNT) against the ancestral strain, with X indicating the median level. Panel C: Live virus plaque reduction neutralization test (PRNT) against ancestral strain with endpoints at 50% ($PRNT_{50}$) with X indicating the geometric mean titer in each group. Panel D: Live virus $PRNT_{50}$ against the Omicron BA.2 subvariant, with X indicating the geometric mean titer in each group. In panels C and D, antibody titers measured at <10 are plotted at 5 on the y-axis.

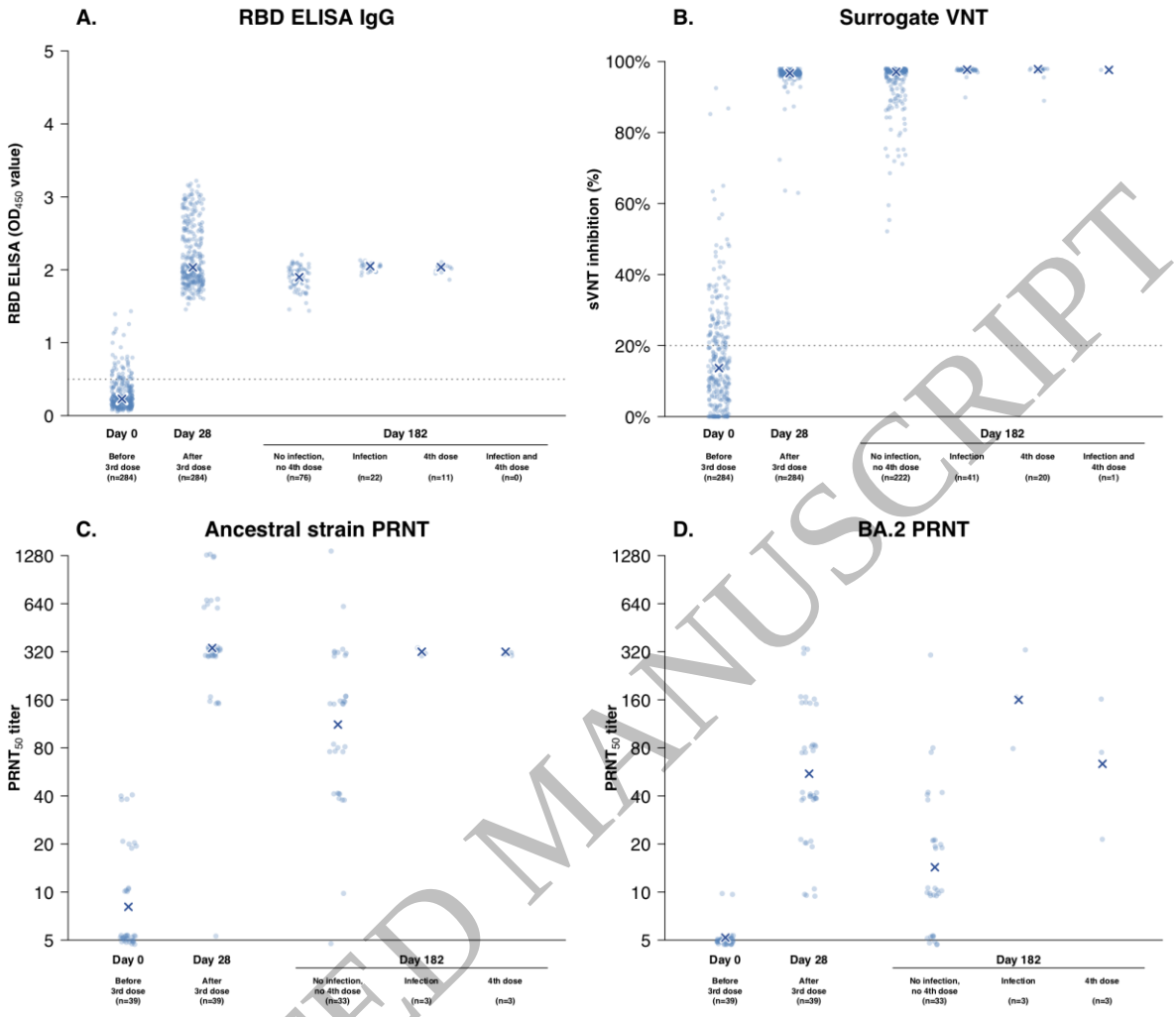

Figure 1
159x136 mm (.77 x DPI)