Delayed interval BNT162b2 mRNA COVID-19 vaccination provides robust immunity

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Brief Communication

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Abstract

Shortages of COVID-19 vaccines have resulted in delayed dosing intervals as a strategy to immunize a greater proportion of the population. The effect of this strategy on vaccine immunogenicity is not well studied. Humoral (anti-RBD levels and neutralization) and cellular immune responses were compared in health care workers receiving two doses of BNT162b2 (Pfizer-BioNTech) vaccines at standard (3-6 week) and delayed (8-12 week) intervals. In the delayed group, anti-RBD antibody titres were significantly enhanced compared to the standard interval group. Neutralizing antibody responses were excellent and comparable in both groups. A slight decrease in Spike-specific polyfunctional CD4+ T-cells expressing interferon- γ and IL-2 as well as monofunctional CD4+ T-cells was seen in the delayed group. Both polyfunctional and monofunctional CD8+ T-cell responses were comparable. Our data suggest that the strategy of delayed second dose mRNA vaccination is not overtly detrimental, and specifically may lead to an enhanced humoral immune response.

Main Text

Multiple vaccine platforms have been investigated and implemented worldwide to impede the spread of the coronavirus disease 2019 (COVID-19), proving extremely successful in the prevention of infection and symptomatic disease ¹. The BNT162b2 mRNA vaccine (Pfizer-BioNTech) has been authorized for administration with a three-week interval between the 2 doses ¹. Similarly, the mRNA-1273 vaccine (Moderna) is approved based on a 4-week dosing interval.

Inequities in global vaccine access have resulted in supply shortages in many jurisdictions. In Canada, a 4-month delay between vaccine doses commenced from March 3rd, 2021, as part of a strategy to accelerate the proportion of the population that receive one dose and achieve higher overall protection in the context of limited vaccine supply ². Delaying the second vaccine dose was controversial, with theoretical concerns of reduced immunogenicity creating selective pressure and facilitating the emergence of vaccine-resistant strains, as well as reduced vaccine effectiveness in prevention of infection and the durability of neutralizing antibody (NAb) responses after one dose of vaccine compared to two doses ^{1,3,4}. A meta-analysis of two doses of the ChAdOx1 nCoV-19 vaccine has supported a delayed interval of 3 months between vaccine doses for improved vaccine efficacy and binding antibody response⁵. Currently, there is limited peer-reviewed published literature examining the influence of delayed interval vaccination with mRNA vaccines, including the BNT162b2 mRNA vaccine, on immunogenicity. In this study, we compared the anti-receptor binding domain (RBD) antibody, serum neutralizing antibody and mono- and polyfunctional CD4+ and CD8+ T-cell immune responses in otherwise healthy health care workers (HCWs) who received two doses of the BNT162b2 vaccine from January 2021 to June 2021 at a standard interval (3-6 weeks) versus a delayed interval (8-12 weeks).

A total of 90 HCWs were enrolled and received vaccination with 2 doses of the BNT162b2 vaccine. Of these, 39/90 (43.3%) received both doses of the vaccine at the standard interval and 51/90 (56.7%) at the delayed interval. Median time to second dose in the standard interval group was 35 days (Interquartile

Range (IQR) 21- 35) and delayed interval group was 88 days (IQR 84 -90). The median age of the cohort was 40.2 years (IQR 34.2 – 51.8) and participants were predominantly female (82/90, 91.1%). Median age was similar between the two groups, 40.3 years in standard and 40.2 years in the delayed interval groups; female sex was also similarly distributed, 31/39 (79.5%) vs 42/51 (82.4%) participants in the two groups respectively. Two participants had detectable anti-RBD antibody prior to vaccination although did not have a history of documented COVID-19. No participant developed symptomatic COVID-19 infection after vaccination over the study period.

There were robust humoral immune responses after two doses of the BNT162b2 vaccine (Figure 1a) in all participants. However, anti-RBD antibody titre was significantly greater (Figure 1a) in the delayed interval group compared to the standard interval group (median 11,363 U/ml (IQR 7,880 – 17,647) compared with 3,420 U/ml (IQR 1,792 – 7765) respectively, $p < 0.0001$). This increase in titre had a positive correlation to time interval between the two doses (Spearman $r=0.538$, $p < 0.0001$). Percent neutralization was determined using a surrogate virus neutralization assay (SVNT) with a positive threshold defined as $\geq 30\%$. Neutralizing antibody response was strongly positive for the majority of participants after two doses of vaccine (Figure 1b) and with no statistical difference in median percent neutralization between the standard and delayed interval groups (median 96.8% (IQR 96.2-97.4) in the standard interval group vs 96.8% (IQR 96.6-97.2); $p = 0.6439$). In the delayed cohort, 25/30 (83.33%) participants were found to have a positive neutralizing antibody after one dose only, which was boosted after the second dose of vaccine in the majority of participants.

Cell-mediated immune responses were determined following stimulation with overlapping spike-protein peptides. Spike-specific polyfunctional CD4+ T-cells, defined by double positivity for interferon- γ (IFN- γ) and IL-2 (Figure 1c), were slightly lower in the delayed interval group compared with the standard interval group (median T-cell frequency 208 (IQR 128-407) vs. 429 (IQR 191-792) cells per 10^6 CD4+ T-cells, delayed vs. standard interval respectively, $p=0.0451$). This decrease in CD4+ T-cell response was correlated with time interval between the two doses (Spearman $r = -0.371$, $p = 0.0073$). However, the proportion of patients above the pre-defined cut-off of 0.01% (or 100 cells per million) was similar in the two groups (97.4% standard vs 84.6% delayed interval, $p=0.1561$). Monofunctional CD4+ T-cells (Figure 1e) also displayed lower frequency in the delayed vaccine group for both IFN- γ positive and IL-2 positive populations ($p=0.031$ and 0.00087 respectively). Polyfunctional CD8+ T-cell responses tended to be low in both cohorts (Figure 1d) and overall only 6/51 (11.8%) participants elicited a vaccine-specific polyfunctional CD8+ T-cell response if a threshold value of $\geq 0.01\%$ was applied. However, CD8+ monofunctional IFN- γ or IL-2 were readily detectable in both groups (Figure 1f), with only a trend towards a more robust IFN- γ response in the standard interval group ($p=0.05$).

Adverse events (AEs) for dose 2 were comparable between the standard ($n=38$) and delayed interval ($n=51$) groups (Figure 2a,b). Specifically, systemic inflammatory events such as fever and chills occurred with comparable frequency in both groups. However, myalgia was reported more commonly in the delayed group (32.0% vs 15.8% respectively). In an analysis of the entire cohort, the severity of adverse events correlated with the degree of anti-RBD response. In those participants who experienced grade 2

events (14/90, 15.6%) with the second dose of vaccine (Figure 2c), median anti-RBD antibody titre after the second dose was 14,617 U/ml (IQR 7,692 – 21,702) compared with 7,980 U/ml (IQR 3,263 – 11,396) in those who experienced only grade 1 or no adverse events ($p = 0.0036$).

Delayed dosing of mRNA COVID-19 vaccines has been a politically controversial issue, based primarily on pragmatic reasoning aimed at mitigation of vaccine shortages, rather than scientific justification. The main findings of this comparative assessment of humoral and cellular immune response in HCWs receiving 2 doses of the BNT162b2 vaccine were as follows: 1) all health care workers, regardless of regular or delayed dosing intervals, produced robust immune responses after two doses of the vaccine, 2) interestingly anti-RBD antibody titres were significantly enhanced by a delayed interval while neutralizing antibody responses were robust in both groups, 3) cellular immune response represented by spike-specific monofunctional and polyfunctional CD4⁺ T-cells were detectable in both groups but may be slightly lower in the delayed vaccination group. Less impact of delayed dosing was apparent upon the CD8⁺ T-cell response. The data presented herein provide scientific rationale to support delayed dosing policies for mRNA vaccines especially since currently there are limited peer-reviewed published data reporting on this.

Concern has been raised that delaying the interval of the 2 doses of vaccine may encourage the development of new SARS-CoV-2 variants in the interim while awaiting the second dose³. This may be off-set, however, by the higher anti-RBD titres achieved in the delayed vaccination group, possibly conferring a more durable response and longer-term protection, as well as potentially increasing the likelihood of sufficient antibody level to confer efficacy against heterologous and partially vaccine-resistant variants. The greater humoral response achieved by delaying mRNA vaccination is consistent with studies of delaying the second dose of vector-based (ChAdOx-1) vaccine⁵.

A significant decrease in the quantity and duration of neutralizing antibodies has been described in the period between the first and second doses of the BNT162b2 vaccine and across all age groups^{3,4}. This may lead to reduced levels of immunity and protection from infection. All HCWs in our study achieved high neutralization levels after 2 doses of vaccine and there was no difference observed between the two groups. The majority of those in the delayed cohort who underwent blood sampling after one dose of vaccine were found to have a positive neutralization assay, although exhibiting a lower percent neutralization compared to post-second dose. None of our participants reported symptomatic COVID-19 infection prior to the second dose.

Our study also suggests that delaying the second dose of BNT162b2 vaccine at an 8-12-week interval may lead to a lower spike-specific CD4⁺ T-cell frequency. Dan et al⁶ described that memory B cells against SARS-CoV-2 spike increased between 1 month and 8 months post-infection, while memory CD4⁺ T cells declined. Similarly, Gaebler et al⁷ observed that by 6-months post-infection, the number of RBD-specific memory B cells remained largely unchanged but displayed greater resistance to RBD mutations and had increased potency. This is likely a result of germinal center reactions in peripheral lymphoid tissues, where follicular helper T-cells help guide processes like somatic hypermutation and affinity

maturation that evolve the humoral response over time. Both mRNA vaccines have demonstrated potent SARS-CoV-2 specific germinal centre responses, even with just a single dose of vaccination⁸. As such, we propose that the memory B-cell repertoire at the time of delayed vaccination may simply be of a greater calibre, leading to higher quantity and/or quality of antibody response. Analogous maturation processes are not known to occur for T-cells, which are thought to contract at a faster rate than B-cells⁹. While we did not measure follicular helper T-cells in our study, we did measure a significant decrease in both monofunctional and polyfunctional CD4+ T-cell frequencies with the delayed immunization protocol. However, no statistical difference was measured between groups with respect to the proportion of individuals with a positive polyfunctional T-cell response greater than the 0.01% threshold, suggesting that delayed immunization is still capable of inducing robust vaccine-specific CD4+ T-cell responses. Whether the difference is clinically relevant is unknown as no cellular correlate of immunity has been identified.

Our study had some limitations. Our cohort was younger and female predominant, both factors can positively influence the immune response¹⁰. However, given similar age and sex distribution in the standard and delayed groups, this would not influence the group comparisons. A strength was the duration of the study and follow-up post vaccination, with all participants observed for 7 months and at least 6 weeks post their 2nd dose of vaccine.

In summary, our study provides scientific rationale to support the implementation of a delayed vaccination schedule if necessary due to limited vaccine supply and suggests that this may be beneficial with respect to humoral immune response. The potentially negative effect of vaccine delay on SARS-CoV-2 specific cellular immune responses, specifically CD4-T-cell responses may perhaps mitigate the humoral immune response benefits. However, further studies of the significance of these findings, including the durability of the immune response over time, correlates of protective efficacy, and immunogenicity as it relates to heterologous variant viruses would be helpful to define optimal dosing interval as well as the necessity/timing of a booster dose of vaccine.

Methods

Patient population and study design

- This was a prospective observational cohort study of healthcare workers (HCWs) recruited from a tertiary, University-affiliated hospital network in Toronto, Canada, who were eligible to receive the two scheduled doses of the BNT162b2 (Pfizer/BioNTech) mRNA vaccine. Potential participants were recruited by email to HCWs working at several hospital sites. Those who completed the two-dose vaccine series between January 4th, 2021 – June 13th, 2021 were included and followed for at least 6 weeks after the second dose. Participants were excluded if they had known previous COVID-19 infection or had an immunosuppressive condition. The local institutional research ethics board approved the study and all participants provided written consent. Delayed dosing for HCW came into effect on March 3, 2021. This allowed for two study cohorts: those who received two doses at the

standard interval (3–6 weeks) and those who received two doses at a delayed interval of > 8 weeks. All participants provided blood samples prior to vaccination and 4–6 weeks after the second dose of vaccine. In the delayed interval group, an extra blood sample was obtained 4 weeks after dose one.

Antibody responses and cell mediated immunity assessment

- The primary outcome was measurement of antibody against the receptor binding domain of the spike protein (anti-RBD) using a chemiluminescent microparticle immunoassay (Roche Elecsys). The lower limit of detection of the assay is 0.4 U/mL, with a positive response indicated as ≥ 0.8 U/mL^{11,12}, although higher levels may be needed for protection against infection. Neutralizing antibodies (NAb) were assessed by a validated SARS-CoV-2 Surrogate Virus Neutralization Test assay (GenScript), according to the manufacturer's specifications, with a threshold of 30% neutralization/inhibition or greater indicating a positive assay. The assay has FDA emergency use authorization and has been used in multiple studies evaluating vaccine immunogenicity^{13,14}.

In a subset of participants who provided additional blood, Spike-specific CD4 + and CD8 + T-cell responses were assessed as previously described¹⁵. Briefly, peripheral blood mononuclear cells underwent Ficoll-based isolation and were cryopreserved in liquid nitrogen until use. A total of 10^6 peripheral blood mononuclear cells (PBMCs) were rested for 2h and subsequently incubated with 15-mer overlapping peptides encompassing the full SARS-CoV-2 spike protein (PepTivator®, Miltenyi Biotec) at a final concentration of 5µg/mL per peptide, along with a CD28/CD49d costimulatory antibody cocktail (BD Biosciences) and a protein transport inhibitor to prevent cytokine release (ThermoFisher Scientific). Cells were fixed, permeabilized and incubated with an antibody cocktail to detect intracellular markers (IFN-γ, and IL-2). PMA/ionomycin was used as a positive control and cell with media alone were used as a negative control. Frequencies of CD4 + and CD8 + T-cells that expressed both cytokines (polyfunctional cells) were measured. Flow cytometry was performed on an LSR II BGRV (BD Biosciences) at the SickKids-UHN Flow Cytometry Facility. The frequency of vaccine-antigen specific T-cells was determined by subtracting the frequencies of polyfunctional T-cells in unstimulated comparators from peptide-stimulated samples. A representative gating strategy is shown in Supplementary Fig. 1. A positive T-cell response was defined as a minimum of three standard deviations above the mean of the background and a minimum of 0.01% was set as the limit of quantitation. A minimum of 100,000 live CD3 + T-cells were required for samples to be included in the flow analysis.

Safety and adverse events

Safety assessments included monitoring through a participant-directed vaccine diary for local and systemic adverse events for 7 days after each injection. Adverse events were categorized by the FDA toxicity grading scale for volunteers in vaccine trials as follows; grade 1 (no interference in daily activities), grade 2 (some interference in daily activities), grade 3 (participants unable to perform daily activities) and grade 4 (potentially life threatening)¹⁶.

Statistical analysis

The primary analysis included post-second dose anti-RBD titres, neutralizing antibody, and spike-specific polyfunctional CD4 + and CD8 + T-cells between the standard and delayed interval groups. These were compared between groups using Mann Whitney U test and a significant p-value was set at 0,05.

Secondary analyses were as follows: (i) correlation between anti-RBD titre and time between doses using Spearman's test; (ii) correlation between spike-specific polyfunctional CD4 + T-cell response and time between doses using Spearman's test; (iii) Proportions of persons with polyfunctional T-cell responses between groups using two-tailed Fisher's exact test; (iv) a comparison between groups of post-second dose monofunctional CD4 + and CD8 + T-cells expressing IL-2 or IFN- γ using Mann Whitney U; (v) the median anti-RBD titer in those with grade 2 events compared to those no or grade 1 events using Mann-Whitney U test. All statistical analysis was performed using SPSS version 29.0 (Chicago, Ill) and Prism GraphPad version 9.1.1.

Declarations

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AUTHOR CONTRIBUTIONS

Study Design: DK, AH, VGH, VHF;

Data Analysis: VGH, VHF

Performance of Study: VGH, VHF, TK, MI, BM-K, VK, AY;

Manuscript Writing: DK, AH, VGH, VHF.

COMPETING INTERESTS STATEMENT

DK has received research grants from Roche, GSK, and advisory fees from Roche, GSK, Sanofi, Merck. AH has received a research grant from Roche and advisory fees from Merck. The remaining authors have no conflicts of interest to declare.

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Figures

Figure 1

Comparative analysis between standard (3-6 weeks) vs. delayed (8-16 weeks) second dose interval in healthcare workers receiving two doses of BNT162b2 vaccine a. Anti-RBD titre (U/mL); b. Percentage neutralizing antibodies. Dotted line represents positivity threshold of 30%; c. Spike-specific Polyfunctional CD4 $^{+}$ T-cell counts per million CD4 $^{+}$ T-cells; d. Spike-specific Polyfunctional CD8 $^{+}$ T-cell counts per million CD8 $^{+}$ T-cells; e. Spike-specific Monofunctional CD4 $^{+}$ T-cell counts per million CD4 $^{+}$ T-cells for IFN- γ and IL-2; f. Spike-specific Monofunctional CD8 $^{+}$ T-cell counts per million CD8 $^{+}$ T-cells for IFN- γ and IL-2. Dotted line in Figure 1c-f represents threshold of 100 per million cells (0.01%). Horizontal lines represent median and interquartile range. Between group comparisons done using the Mann-Whitney test.

Figure 2

Adverse events to second dose in standard (3-6 weeks) vs. delayed (8-16 weeks) interval groups. a. Local adverse events; b. Systemic adverse events; c. Anti-RBD titre in participants who had Grade 2 adverse events vs. no or Grade 1 adverse events. Horizontal lines represent median and interquartile range. Anti-RBD titer analyzed using Mann-Whitney test.

Supplementary Files

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- [SupplementaryFigure1.docx](#)