Real world immunogenicity of the 3rd BNT162b2 dose among elderly

Keren Asraf[1], Mayan Gilboa[2,3], Michal Mandelboim[3,4], Victoria Indenbaum[4], Yaniv Lustig[3,4], Carmit Cohen[5], Galia Rahav[2,3], Sharon Amit[6], Hanaa Jaber[5], Ital Nemet[4], Limor Kliker[4], Erez Bar-Haim[7], Ella Mendelson[3,4], Carmit Rubin[5], Ram Doolman[1], Gili Regev-Yochay[3,5] and Yitshak Kreiss[3,8]

[1]The Dworman Automated-Mega Laboratory, Sheba Medical Center, Tel-Hashomer, Ramat-Gan, Israel; [2]The Infectious Disease Unit, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel; [3]Sackler School of Medicine, Tel-Aviv University, Tel Aviv, Israel; [4]Central Virology Laboratory, Ministry of Health, Tel-Hashomer, Ramat Gan, Israel; [5]The Infection Prevention & Control Unit, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel; [6]Clinical Microbiology, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel; [7]Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Nes Ziona, Israel; [8]General Management, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel

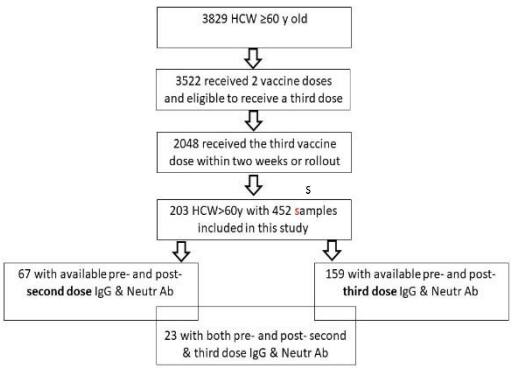
Introduction

The introduction of the BNT162b2 mRNA Covid-19 vaccine has been a tipping point in harnessing the COVID-19 pandemic. In Israel where the vaccine rollout was early and efficient, an impressive decline in new cases was achieved after a little over two months since the initiation of the vaccination campaign, with a marked decrease in severe cases and hospitalizations. Facing the emergence of the delta variant six months later, it was apparent that this effect has been subsided with increasing cases especially among the elderly and those vaccinated earlier, suggesting waning of vaccine immunity.

Following many discussions and accumulating data showing a decay in humoral immunity amongst vaccinated population within 6 months, decreased effectiveness of the vaccine with time and the recently reported correlation between humoral immunity and breakthrough infections, a third dose of BNT162b2 was approved by the Israeli MOH to individuals over 60 years. Here, we present the early immune response (within 14 days) as well as initial safety data following the third dose.

Methods:

Study design and population



SARS-COV-2 RBD IgG testing

Serum was tested for IgG antibodies against SARS-CoV-2 RBD using the commercial automatic chemiluminescent microparticle immunoassay (CMIA) SARS-CoV-2 IgG II Quant (Abbott, IL, USA) according to manufacturer's instructions.

SARS-CoV-2 pseudo-neutralization assay

SARS-CoV-2 pseudo-virus neutralization assay was done using a green fluorescent protein reporter-based pseudotyped virus with a vesicular stomatitis virus backbone coated with SARS-CoV-2 spike protein. A fourfold increase or above in neutralization titers between two consecutive samples was considered a significant increase.

IFN-γ ELISpot assay

PBMC collected by Ficoll were either frozen with CTL-Cryo ABC Media Kit (CTL, Germany) according to the manufacturer's protocol and kept in -70oC or used Fresh. Frozen PBMC were thawed one day prior to analysis, washed and left for overnight rest in CTL test medium (CTL, Germany). IFN-γ-secreting cells were enumerated using Elispot IFN-γ kits (IFN-γ kit, AID Autoimmun Diagnostika GmbH, Strassberg, Germany or IFN-γ FluoroSpot kit ,CTL, Germany)) according to manufacturer instructions. For antigen stimulation, 50 µl of SARS-CoV-2 peptide pools (S-complete, Miltenyi Biotech, or PM-WCPV-S, JPT Peptide Technologies GmbH, Berlin, Germany) were used. Test medium was used as negative control and Phytohaemagglutinin (PHA) was used as positive control. IFN-γ-secreting cells frequency was quantified using the ImmunoSpot S6 Ultimate reader (CTL, Germany) or the AID ELISpot Reader (Strassberg, Germany). The unspecific background (mean SFU from negative control wells) was subtracted from experimental readings.

SARS-CoV-2 micro-neutralization assay

VEROE6 cells at a concentration of 20*103/well were seeded in sterile 96-wells plates with 10% FCS MEM-EAGLE medium, and incubated at 37°C for 24 hours. One hundred TCID50 of WT (hCoV19/Israel/CVL-45526-ngs/2020) and B.1.617.2 (Delta, hCoV-19/Israel/CVL-12804/2021) SARS-CoV-2 isolates were incubated with inactivated samples diluted 1:8 to 1:16384 in 96 well plates for 60 minutes at 33ºC. Virus serum mixtures were added to the VERO-E6 cells and incubated for five days at 33ºC after which Gentain violet staining (1%) was used to stain and fix the cell culture layer. Neutralizing dilution of each serum sample was determined by identifying the well with serum dilutions without observable cytopathic effect. A dilution equal to 1:10 or above was considered neutralizing.

Statistical analysis

Log transformation was calculated for IgG and neutralizing antibody results. To compare paired pre- and post-second or third dose antibody levels, as well as post-second to post-third dose levels, log of antibody levels were compared using Wilcoxon sign rank test. Data analysis was performed using SAS for UNIX 9.4.

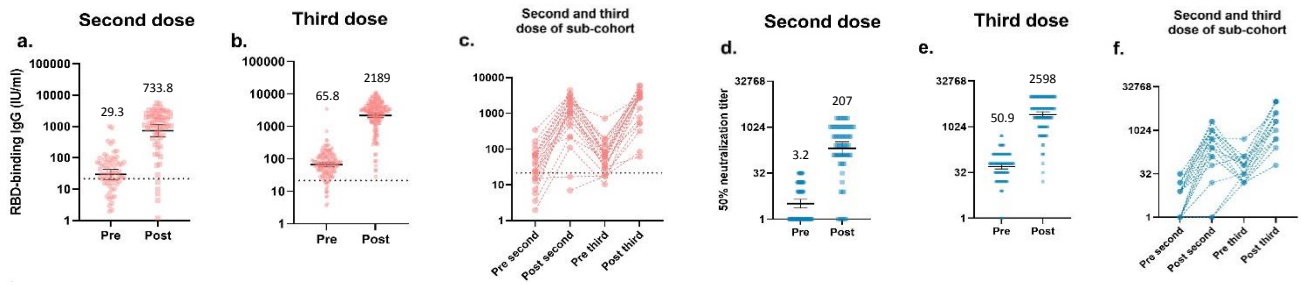


Figure 1: Paired RBD binding IgG and 50% neutralization titer prior to and one to two weeks after receiving second and third dose of BNT162b2 mRNA Covid-19 vaccine. **a.** IgG pre and post second dose of vaccine. **b.** IgG pre and post third dose of vaccine. **c.** IgG levels before and after second and third dose of a cohort of 23 participants. **d.** neutralization titer pre and post second dose of vaccine. **e.** neutralization titer pre and post third dose of vaccine. **f.** neutralization titers before and after second and third dose of a cohort of 23 participants.

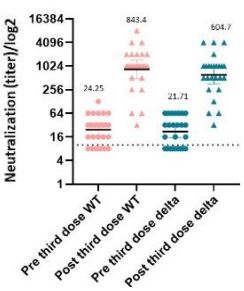


Figure 2: Micro neutralization assay of sera of vaccinated individuals against wild type, and delta variant viruses prior to and after receiving the third dose of BNT162b2 mRNA Covid-19 vaccine.

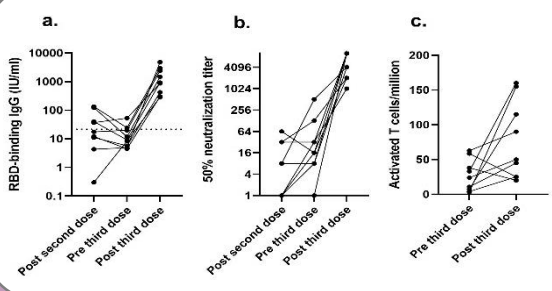


Figure 3: Results of RBD-binding IgG, 50% neutralization titers, and T cell activity of 9 participants who responded poorly to the two first doses of the BNT162b2 vaccine. **a.** RBD-binding IgG including the levels after the second dose of vaccine, levels prior to third dose and levels one week after third dose. **b.**50% neutralization titer of these participants after second dose, before third dose and one week after. **c.** IFN-γ secretion frequencies following T cell activation with spike protein before and after third dose.

Summary and conclusions

- Within one week after receiving the third dose of the BNT162b2 mRNA COVID-19 vaccine, a significant response was observed in all participants, even those who did not respond to previous doses
- The response to the third dose was greater than the response to the second dose
- The humoral and T-cell response was significant increase following the third vaccine dose, in a sub-group of low-responders
- The neutralization of the wild-type the the delta variant was effective and comparable following the third dose

In this study we show that in a real life setting the third dose of the BNT162b2 vaccine produces high immunogenicity against wild type strain and the delta COVID-19 variant