**Brief Communication**

**The humoral response to breakthrough infection or fourth BNT162b2 vaccine dose** **in patients with cancer**

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**Keywords**: SARS-CoV-2, COVID-19, 4th vaccine dose, Second Booster BNT162b2, Cancer, Immunoglobulin-G (IgG)

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**Abstract**

Since January 2022 in Israel, high-risk populations with underlying health conditions, such as oncology patients, are advised to be vaccinated with the 4th-BNT162b2-dose. Nevertheless, no data concerning its efficiency and necessity in oncology patients is yet available. We monitored vaccine-induced immunity of oncology patients under systemic anti-cancer therapy, vaccinated with 4th-BNT162b2-dose and compared anti-Covid-19 IgG levels before and after 4th-BNT162b2-dose to patients experiencing breakthrough-infection and to control group of patients choosing to avoid it. Concurrently to a significant waning of IgG levels in the control group, a considerable elevation in IgG titer was observed in vaccinated patients as an indication of 4th-BNT162b2-dose capacity to boost anti-Covid-19 IgG response among cancer patients. Surprisingly, IgG levels of patients experiencing breakthrough-infections were substantially higher than those vaccinated with 4th-BNT162b2-dose. Our findings indicate that humoral response by oncology patients, generally considered immunocompromised, is significantly more potent after recovery from Covid-19 infection than the 4th-BNT162b2-dose.

**Introduction**

Since January 2, 2022, the Israeli Ministry of Health has advised high-risk individuals, such as oncology patients, to be vaccinated with a second BNT162b2 vaccine booster of the 4th-BNT162b2-dose. However, no data assessing the efficiency and necessity for this 4th-BNT162b2-dose in oncology patients is still not available1. We examined the impact of Covid-19 vaccine breakthrough infection compared to vaccine booster-induced immunity and compared it to the anti-Covid-19 immunoglobulin-G (IgG) levels of oncology patients vaccinated only with the 3rd- BNT162b2-dose as a control group.

**Materials and Methods**

**Participants and Design**

Study participants included oncology patients with solid tumors on active anti-cancer therapy, receiving intravenous treatment administered at the oncology infusional ambulatory unit, Emek Medical Center. All donors enrolled in the study were 18 years or older, vaccinated with the 3rd-BNT162b2-dose (BioNTech Pfizer, Manhattan, New York, United States), and eligible for the 4rd-BNT162b2-dose. Patients with previous confirmed records of COVID-19 infections or active autoimmune disease were excluded. After signing a written informed consent, blood samples were first taken (up to 30 days) before the scheduled 4rd-BNT162b2-dose. Second blood tests (IgG blood test-2) were taken from donors 30-90 days after a specific breakthrough infection (166.00 days from dose-3 (IQR[139.00, 173.00]) or the 4th-BNT162b2-dose administration (166.00 days from dose-3 (IQR[161.00, 177.50])). To adjust and match the IgG blood test-2 sampling times of the control group to the other two study groups, we scheduled IgG blood tests-2 of all control group donors to 160.00 days from dose-3 (IQR[148.25, 170.50]) (**Table-1**). Data was collected and distributed according to three categories: (1) patients reporting Covid-19 infection (Covid-19 infection) and (2) patients vaccinated with the 4th-BNT162b2-dose (4th-BNT162b2-dose) between blood sampling. Patients choosing to avoid 4th-BNT162b2-dose and did not experience Covid-19 infection served as (3) reference group (Control).

**Immunoassay**

Anti-Covid-19 IgG titers were quantified by the SARS-CoV-2 IgG serological immunoassay (Alinity, Abbott Core Laboratory, Abbott Park, Illinois, United States)2. According to the manufacturer's protocol limitation of the procedure, minimum values for seropositive detection were set at 50 arbitrary units per milliliter (AU/ml), and maximum detection values were set at 40,000 AU/ml2.

**Statistical Analysis**

To test groups' differences, we used the Wilcoxon Rank U test (this is equivalent to the Mann-Whitney U test when there are only two groups). For the post-hoc test (**Figure-1b**), we used a one-sample t-test to determine whether the IgG titers (2nd blood test) of the control and the vaccinated group were different from the constant value (of 40,000 Au/ml) associated with the IgG titers of the Covid-19 infection group. Statistical significance was set at a 0.05 threshold value. Because of the limited sample size, we may incur Type II errors (false negative) when testing differences between groups. Therefore, inferences and generalizations based on the results of this study should be made with caution.

**Results**

To assess the humoral immunity of oncology patients under active systemic anti-cancer therapy to the 4th-BNT162b2-dose, we followed donors, fully vaccinated with the 3rd-BNT162b2-dose, participating in our recent study, monitoring vaccine-derived immunity after the 3rd-BNT162b2-booster-dose2. Upon study initiation, approximately 60% of the previous 154 participants were excluded mainly from Covid-19 infections or chose not to be immunized with the 4th-BNT162b2-dose. All remaining donors (n=63) were approached when almost 70% were willing to participate in further serological tests (n=42). The study included 21 (50%) males when participants ages ranged from 63 to 68 (**Table-1**). All subjects were seropositive to anti–SARS-CoV-2 shortly after the 3rd-BNT162b2-boost, showing a mean IgG titer of 14,813 AU/ml2. A subset of patients (n=9, 21.4%) experienced Covid-19 breakthrough infection after the 3rd dose (days after 3rd dose to infection=136.6 days).Another group of patients (n=11, 26.19%) chose to be administered the 4th-BNT162b2-dose and did not experience Covid-19 infection (days to 4th-BNT162b2-dose =131 days). As a control, we included a group of patients (n=22, 52.38%) with no record or known ailments who did not receive the4th-BNT162b2-dose.About 63-68 days after the 3rd-BNT162b2-dose (days from dose-3 to blood-test-1, *P* = 0.171) (**Table-1**), we quantified the IgG levels against the spike receptor-binding domain (RBD) of SARS-CoV-2 by serological immunoassay for all study donors (IgG blood-test-1) (**Table-1**). All second blood tests assessing anti-Covid-19 IgG (IgG blood test-2) after a specific event were taken up to 30 to 90 days post-breakthrough infection or the 4th-BNT162b2-dose administration. Simultaneously with the other two study groups (160- 166 days from dose-3 for study donors), anti-Covid-19 IgG titers were also assessed in a control group (days from dose-3 to blood-test-2, *P* = 0.169). No significant difference between levels of the antibody titers at baseline levels at blood-test-1 (*P = 0.647*) (**Figure-1a** and **Table-1**). Control group anti-Covid-19 IgG levels sharply drop from to 5379.95 Au/ml (interquartile range (IQR) [2030.95, 25999.52]) to 2314.00 Au/ml (IQR [459.00, 5540.05]) at blood-test-2 (*P<0.001*) (**Figure-1a**), as an indication of the waning short-lived covid-19 immunity3. Patients who experienced Covid-19 vaccine breakthrough infection showed a dramatic and significant elevation of anti-Covid-19 IgG titer from 7050.50 Au/ml (IQR[3329.50, 11514.50]) to 40000.00 Au/ml (IQR [39873.00, 40000.00])(*P<0.001*) (**Figure-1a**) and compared to control group at blood-test-2 (*P<0.001*) (**Figure-1b**). At the same time, among patients vaccinated with the 4th-BNT162b2-dose, we observed a significant but modest increase of anti-Covid-19 specific IgG from 5379.95 Au/ml (IQR[2030.95, 25999.52]) to 22914.00 Au/ml (IQR [16562.00, 40000.00] (*P = 0.033*) (**Figure-1a**) compared to control at blood-test-2 (*P<0.001*) (**Figure-1b**), indicating the effectiveness of 4th-BNT162b2-dose4 and it's capacity to boost anti-Covid-19 IgG levels5 among patients with cancer. Surprisingly, when comparing patients vaccinated with the 4th-BNT162b2-dose versus those who experienced Covid-19 vaccine breakthrough infection, the mean of anti-Covid-19 IgG levels of the infected group was considerably and significantly (*P* = 0.0050) higher than the vaccinated group (**Figure-1b**).

**Discussion**

Although limited by the small sample size, our data indicate that oncology patients; who are generally considered immunocompromised and are at a higher risk of infection with severe outcomes1; when fully vaccinated with the 3rd-BNT162b2-dose elicited a vigorous and superior humoral immune response after exposure to SARS-CoV-2 infection, then achieved by the 4th-BNT162b2-dose consistent with the observations in healthy individuals experiencing repeated SARS-CoV-2-S hybrid exposure6. At the point of comparative measurement, anti-Covid-19 IgG levels were significantly lower in oncology patients vaccinated only by the 3rd-BNT162b2-dose compared to patients vaccinated with the 4th-BNT162b2-dose or exposed to naturally acquired immunity (e.g., Covid-19 infection). It appears that immunity against Covid-19 rapidly wanes in patients that do not experience any exposure to SARS-CoV-2 spike protein (either by infection or by additional booster).

Indeed, recent findings indicate that exposure to covid-19 by breakthrough infection may better protect against hospitalization, effectively prevents reinfection7, improves survival regardless of covid-19 variants7, and elicits potent, more durable, and broad neutralizing antibody responses6,8, even against the Omicron variant9. As future SARS-CoV-2 variants may emerge and the population will keep acquiring enhanced variant and age-independent SARS-CoV-2 immunity9, oncology patients infection records should be taken into account when evaluating the risks and outcomes of new BNT162b2 booster regimen.

Further BNT162b2 booster protective effectiveness and the durable immunity after the 4th-BNT162b2-dose in cancer patients should be compared to the general population to assess whether individuals recovering from Covid-19 infection are indeed better protected from reinfection10.

**Acknowledgments**

We thank Ms. Tami Appelbaum for her significant help with linguistic and manuscript editing.

**Conflict of interest**

All authors declare no conflict of interest

**Authors contribution**

BSG was responsible for donor recruitment and patient monitoring. RO and CR supervised and managed clinical data collection and IgG immunoassays. SCP, CI, and BSG performed the statistical analysis and documentation, generated the tables and figures, and wrote the manuscript. CI and BSG conceived, designed, supervised, and sponsored the study.

**Data Availability Statement**

The data used and analyzed in the study is available from the corresponding authors upon reasonable request.

**Abbreviations**

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)

Coronavirus Disease 2019 (COVID-19)

Immunoglobulin-G (IgG)

**Funding**

Research in our laboratory is funded by the Ministry of Health (Jerusalem, Israel) grant number: 3000015198 and the Israeli Cancer Association grant number 2020002

**Ethical Statement**

All study donors signed an informed consent form included in the study protocol, which had been authorized and approved by the Institutional Ethics Committee (**0133-21-EMC**). The study was designed and conducted according to the guidelines and rules of the Helsinki declaration for human medical experiments ethical code.

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**Figure legends**

**Figure-1: Assessment of specific anti- SARS-CoV-2 IgG levels of fully vaccinated (Third Dose) oncology patients compared to patients receiving the 4th-BNT162b2-dose or experiencing a breakthrough infection. (A)** Quantification of IgG levels against the spike receptor-binding domain (RBD) of SARS-CoV-2 in control (vaccinated only with Third Dose) (n=22), oncology patients after the second BNT162b2 booster (4th-BNT162b2-dose) (n=11) or patients who experienced covid-19 breakthrough infection (Covid-19 infection) (n=9). Blood test-1 and blood test-2 indicate IgG levels before and after events. **(B)** Levels of IgG anti-SARS-CoV-2 antibodies measured in blood test-2 when the median and interquartile range (IQR) are indicated (**See also Table-1**). Significant differences (p < 0.05) are indicated using horizontal solid lines.

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