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**Manuscript type: Letter**

**Assessment of immune response to anti-COVID-19 booster in patients with advanced cancers and medical cannabis users**

**Evaluation of immune response to anti-COVID-19 booster in oncology patients and chronic medical cannabis users**

**Equivalent and adequate immune response of patients with advanced cancers and chronic medical cannabis users to anti-COVID-19 vaccine booster**

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Letter to the Editor

On 29 Jul 2021, the Israeli Ministry of Health was the first to approve the third anti-COVID-19 vaccination (BNT162b2 booster dose) worldwide, leading to a sharp daily drop in diagnosed positive COVID-19 cases and mortality rates [1]. Until the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) Omicron variant emerged, the daily diagnosis of positive COVID-19 cases resulting in hospitalization due to severe acute respiratory failure remained minimal, with the vaccine-derived protection against SARS-CoV-2 infection achieved by the BNT162b2 booster dose. The booster dose increases Omicron neutralization efficiency approximately a hundred-fold compared to individuals receiving only the second dose, providing significant protection against infection [2]. Due to their immunocompromised condition, cancer patients may be more susceptible and generally more vulnerable to infections. Indeed, due to the chronic weakening of their immune system, cancer patients are at higher risk of developing severe clinical outcomes from SARS-CoV-2 infection, and are associated with an increased risk of morbidity and mortality [3]. Cancer patients treated with anticancer drugs or undergoing major surgery have double the risk of developing a severe illness, hospitalization and death due to COVID-19 [3]. Recent research studies indicate that anti-COVID-19 immunity decreases over time, and that boosters can positively potentiate anti-COVID-19 immunity. Studies of cancer patients who received the second BNT162b2 booster dose indicated a pronounced lag in antibody production compared to controls, yet the seroconversion rate tested four weeks after administration was comparable[4]. No adverse effects or interaction between immunoglobulin G (IgG) levels and active anticancer therapies such as chemotherapy or radiation were reported [4, 5]. Early reports of cancer patients receiving the third BNT162b2 booster dose indicated efficient and robust potentiation of anti-COVID-19 immunity measured over a short period (4 weeks after administration) post-BNT162b2 booster dose, and neither gender nor chemotherapy status were associated with higher antibody levels [6]. Though firmly supporting the BNT162b2 booster dose for actively-treated cancer patients, significantly lower pre-booster and post-booster antibody concentrations were noted (1 month after administration) in oncology patients compared to the control group [7].

Cannabis may potently suppress humoral immunity and antigen-specific antibody production since natural or synthetic tetrahydrocannabinol (THC) derivatives can hinder humoral and cell-mediated immunity [8]. The American Society of Clinical Oncology (ASCO) estimated that by 2021, 20% to 40% of patients with cancer were consuming some form of cannabis either during or after treatment. Due to its potent immunosuppressive properties, no prior reports of anti-COVID-19 antibody production among cannabis users, and its consumption by oncology patients, we see an urgent and immediate need to test the effect of cannabis consumption on anti-COVID-19 immunity following BNT162b2 booster dose vaccination.

 We monitored humoral immunity after the BNT162b2 booster dose to assess cancer patients' vaccine-derived antibody production compared to non-oncology donors. Unlike recent BNT162b2 booster dose reports measuring anti-COVID-19 IgG immediately after administration (3-4 weeks), and before the level of total antibodies and avidity fully increase (from 30 to 120 days), our measurements were taken between 31 to 120 days after the BNT162b2 booster dose, after a full IgG avidity maturation (high-affinity IgG occurrence) humoral response. To enable an appropriate uniformed, matched comparison representation between different groups (i.e., oncology vs. non-oncology donors, and users vs. non-users), we assessed the anti-COVID-19 antibody titer of all samples using a unified, standardized authorized immunoassay.

We assessed IgG titers of oncology versus non-oncology donors as well as cannabis users vs. non-users (**Supplementary Table 1**). A total of 154 participants were grouped into: oncology (n=62); oncology + medical cannabis use (n=25); non-oncology (n=46); non-oncology + cannabis users (n=21); mean age 61 years [IQR25=53, IQR75=71]; females n=85 (55%).Within the oncology group, n=60 were metastatic and n=27 had localized cancer; the most frequent treatment was chemotherapy (n=47), followed by chemotherapy and biological treatment (n=11). Co-morbidities among oncology patients were equally distributed between cannabis users and non-users. Among the cannabis users, the mean daily dosage was 1 g (30 g monthly prescription) (**Supplementary Table 1**).Each individual was tested for IgG levels at a single time point (between day 31 and day 122 after BNT162b2 booster dose (mean 73 days [IQR25=63, IQR75=73]).

Results show that mean IgG titers were equivalent over all the groups considered in this study (**Supplementary** **Table 1** and **Figure 1A**). Overall, donors’ health conditions (i.e., oncology vs. non-oncology) and cannabis use (i.e., users vs. non-users) were not significant factors of variance for IgG titers (two-way ANOVA for cannabis use p=643, health conditions p=681, interaction between cannabis use and health conditions p=0.09). We also tested for other possible sources of IgG variance, specifically treatments, stage and type of cancer. We found no significant association between these variables and IgG titers. Finally, no significant correlation between IgG titers and time of blood sampling was found (**Figure 1B**).

Next, we dichotomized all donors according to IgG titers. As a threshold, we defined IgG=4000 AU/ml, which is 10% of the IgG maximum titer value. Therefore, the groups are: BNT162b2 booster dose low responders (IgG<4000 AU/ml) and high responders (IgG >4000 AU/ml) (**Supplementary Table 2**). Since low responders may be at higher risk of COVID-19 infection due to the low anti-COVID-19 IgG titers, we tested whether this group is associated with any distinct CBC features. Blood samples were taken before the BNT162b2 booster dose (designated as time-2) and again after booster administration (designated as time-3).We found a significant difference in the levels of hemoglobin (HB, p=0.039), white blood cells (WBC, p=0.046), neutrophils (Neu, p-<0.001) and monocytes (Mono, p=0.043) at time-3 (only after BNT162b2 booster dose) between low and high responders. Notably, the levels of eosinophils (EOS) were the only CBC parameter showing a significant difference before the BNT162b2 booster dose (p=0.015) and a borderline significant difference after the BNT162b2 booster dose (p=0.088) (**Figure-1C** and **Supplementary Table 3, Supplementary Table 4)**. Although no rationale is yet presented, as considerable support for our findings, hemoglobin disorders, chronic iron deficiency and anemia were recently highlighted as possible determinants affecting humoral response against COVID-19. Nonetheless, our findings show that these common blood test variables correlate significantly with anti-COVID-19 IgG levels after booster administration. Even though data are yet minimal and largely unexplained, low eosinophil counts or eosinopenia of COVID-19 patients correlate with critical disease progression, predicting transfer to intensive care unit among elderly COVID-19 patients, suggested as a marker to indicate a re-infection of patients who had previously had COVID-19 and, most importantly, higher COVID-19 mortality rates [9, 10].

In contrast to earlier studies reporting antibody production at early time points, our results indicate that vaccine-induced IgG production is equally effective in cancer patients and control groups. We also provided the first assessment of cannabis consumption by oncological patients and non-oncological individuals on anti-Covid-19 vaccination and demonstrated no significant impact or interaction with anti-Covid-19 IgG production. Thus, considering that cannabis consumption is highly prevalent among cancer patients, its safety concerning anti-Covid-19 vaccination is of great significance. Finally, our report also uncovers an unexplained link between circulating eosinophil levels and the immune response to Covid-19, which deserves further attention and additional studies.

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**Figure Legends**

**Figure 1** – **IgG titer levels and CBC parameters over various groups**. (**A**) IgG titers (AU/ml) in relation to two factors of variance, cannabis use (user, non-users) and health conditions (non-oncology, oncology). On each box plot, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Means are plotted individually using the 'full dots' marker symbol. (**B**) Incidence (%) of high responders (Ig>4000) over time (days from vaccination to the measurement of anti-COVID-19 immunoglobulin G (IgG)). (**C**) Correlation of CBC levels and cell counts, in relation to two factors of variance, IgG response (high responders >4000 vs. low responders <4000) and time of measurement (time-2 before 3rd dose vs. time-3 after 3rd dose). Each box represents a different tested blood component. The left side of each box shows measurements before the 3rd dose (time-2), and the right side shows measurements after the 3rd dose (time-3). In each box plot, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Means are plotted individually using the 'full dots' marker symbol.

**Abbreviations**

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

Coronavirus Disease 2019 (COVID-19)

Immunoglobulin-G (IgG)

Tetrahydrocannabinol (THC)

Complete Blood Counts (CBC)

Eosinophils (EOS)

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**Author contributions**

RO, AA, KM, AD, EH and RC performed CBC blood processing analysis and documentation. KM, SH, YS, MC, AAM and BSG were responsible for donor recruitment, monitoring patients, and collecting and assembling clinical data and blood samples. SCP, CI and BSG performed statistics and data analysis, generated the figures and wrote the manuscript. CI and BSG conceived, designed, supervised and sponsored the study.

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**Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

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).

**Consent for publication**

All authors give consent for the publication of the manuscript.

**Competing interests**

The authors declare no competing interests.

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