Original Research

Seropositivity and neutralising antibodies at six months after BNT162b2 vaccination in patients with solid tumours

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Abstract   *Aim:* Patients with cancer are at an increased risk for severe coronavirus disease of 2019. We previously reported initial findings from a single centre prospective study evaluating antibody response after BNT162b2 vaccine, showing that adequate antibody response was achieved after two doses, but not after one, in patients with cancer vaccinated during anti-cancer therapy. Herein, we report a follow-up study, evaluating antibody response six months after the second vaccine dose.

Methods: The study included patients with solid tumours undergoing anticancer treatment, and immunocompetent health-care workers serving as controls. Serum titres of the receptor-binding domain (RBD) IgG and neutralising antibodies (Nabs) were measured approximately six months after the second vaccine dose. Complete blood count values were collected and evaluated as predictors for antibody response.

Results: The analysis included 93 patients with cancer (66.7% metastatic). Six months after the second vaccine dose (mean 176 ± 20 days), seropositivity rate among patients and controls was 83.9% versus 96.3% (p = 0.0001), respectively. Median RBD-IgG titre was lower among patients compared with controls (2.3 versus 3.2, p = 0.0002). Among seropositive individuals,

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median Nabs titre was similar between patients with cancer and controls (p = 0.566). Among patients with cancer, lymphocyte and neutrophil counts were not correlated with either RBD-IgG or Nabs titres.

Conclusions: Seropositivity rates and RBD-IgG titre at six months after second BNT162b2 vaccine dose are lower among patients with cancer compared with healthy controls. However, Nabs titre is similar, suggesting a comparable protection among seropositive individuals. Lymphocyte count is not predictive of antibody response.

1. Introduction

Patients with cancer are at a significantly increased risk of severe morbidity and mortality from coronavirus disease of 2019 (COVID-19) [1–9]. In a previous report, we showed that an adequate antibody response was achieved after two doses of BNT162b2, but not after one, in patients with cancer vaccinated during anticancer therapy, and at lower seropositivity rates compared with healthy controls [10], in accordance with additional reports [11–15]. Conflicting data came from follow-up studies showing that seropositivity rates among patients with cancer compared with healthy controls were lower at four months after the second vaccine dose [16], but were similar at six months [17]. Additionally, a third vaccine dose, given six months after the second vaccine dose, was shown to increase antibody levels in patients with cancer [18,19].

Several previous studies have evaluated possible predictors of seropositivity after BNT162b2 vaccination in patients with cancer during anticancer therapy, suggesting that low lymphocyte counts are associated with lower seropositivity rates [20,21].

Here, we describe the efficacy of BNT162b2 vaccination of actively treated cancer patients at six months after the second vaccine dose. Our aim was to evaluate the rate of seropositivity and neutralising antibodies titres, and to assess complete blood count values as possible predictors for antibody response.

2. Methods

2.1. Study design and participants

Cancer patients who are actively treated in our institution were vaccinated with BNT162b2, regardless of treatment type, disease stage, performance status, or life expectancy. Study period was between 27th December 2020 and 23rd August 2021. Patients infected with SARS-CoV-2 before or during the study period were excluded. Two doses of the BNT162b2 vaccine (Pfizer, New York, USA and BioNTech, Mainz, Germany) were administered, 21 days apart. Patients were actively screened for the vaccine-induced antibody response approximately six months after the second vaccine dose. We matched the case samples with control samples according to age, sex, the interval between the second vaccine dose and serologic testing, and comorbidities (hypertension, diabetes, heart disease, lung disease, and autoimmune disease). Controls were immunocompetent healthcare workers with no history of SARS-CoV-2 infection who tested for antibody response approximately six months after the second vaccine dose. Medical records were reviewed for results of complete blood counts before each vaccine dose and after the second vaccine dose. Written informed consent form (ICF) was obtained from all participants. The Institutional Review Board approved the study protocol and ICF.

2.2. Clinical data extraction

Relevant clinical data was retrieved from electronic medical records of cancer patients and included age, gender, body mass index (BMI), cancer type, diagnosis date, and cancer stage (i.e. local or metastatic). Comorbidities included hypertension, diabetes mellitus, cardiac disease, lung disease, and autoimmune disease. Anticancer therapies were classified as chemotherapy, immunotherapy, biological targeted therapy, hormonal therapy, and radiation, given either alone or in combinations.

2.3. Serology assays

Samples were evaluated with an enzyme-linked immunosorbent assay (ELISA) that detects IgG (Immunoglobulin G) antibodies against the RBD (receptor binding domain) of SARS-CoV-2 [22]. ELISA index value below 0.9 was considered negative, between 0.9 and 1.1 equivocal and equal or above 1.1 positive. Samples that were positive for RBD-IgG were tested for Nabs. A SARS-CoV-2 pseudo-virus neutralization assay was performed using a propagation-competent VSV-spike similar to that previously published [23] (kindly provided by Gert Zimmer, University of Bern, Switzerland). Sera not capable of reducing viral replication by 50% at a 1:8 dilution or below were considered non-neutralising. Negative RBD-IgG samples were not

tested for Nabs, since these have previously been shown to yield negative Nabs tests.

2.4. Statistical methods

Continuous variables are presented as mean and standard deviation or as geometric mean (GMT) and 95% confidence interval (CI). Categorical variables are presented as percentages. For GMT calculation, negative Nabs (=0), missing Nabs (only if RBD-IgG negative), were counted as titres of 2. Spearman's correlation was drawn to evaluate correlation between lymphocyte/neutrophil count and RBD-IgG/Nabs titres among patients with cancer. Differences between groups were assessed using chi-square test and t-test, for categorical and continuous data, respectively. A p-value of < 0.05 was considered statistically significant. Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Patient characteristics

Demographic and clinical characteristics of patients with cancer and controls are shown in Table 1. Baseline characteristics were well balanced between the two groups.

Cancer diagnosis and treatments are detailed in Table 2. Cancer diagnosis included gastrointestinal malignancies in 40 (43.0%) patients, breast cancer in 23 (24.7%) patients, lung cancer in 9 (9.7%) patients, melanoma in 10 (10.8%) patients, genitourinary malignancies in 7 (7.5%) patients, and 4 (4.3%) patients had other tumors (i.e. brain, thymoma, endometrial, and

Table 1
Demographic and clinical characteristics of patients with cancer and controls.

	Patients with cancer (N = 93)	Controls (N = 186)	p-value
Gender N (%)			
Female	56 (60.2)	110 (59.1)	0.863
Male	37 (39.8)	76 (40.9)	
Age mean ± SD	60.8 ± 12.5	61.1 ± 11.1	0.827
BMI mean ± SD	25.7 ± 5.1	26.8 ± 4.7	0.136
Days after second vaccine dose			
Mean ± SD	176.2 ± 20.1	175.5 ± 19.6	0.773
Median (IQR)	178.0 (167.0−189.0)	172.0 (168.0−194.0)	0.547
Comorbidities N (%)			
Hypertension	23 (24.7)	48 (25.8)	0.846
Diabetes	12 (12.9)	26 (14.0)	0.805
Cardiac disease	13 (14.0)	26 (14.0)	1.000
Lung disease	8 (8.6)	16 (8.6)	1.000
Autoimmune	4 (4.3)	8 (4.3)	1.000

BMI, body mass index; SD, standard deviation; IQR, interquartile range.

Table 2
Cancer diagnosis and treatment characteristics.

	Patients with cancer (N = 93)
Cancer type N (%)	
Gastrointestinal	40 (43.0)
Breast	23 (24.7)
Lung	9 (9.7)
Melanoma	10 (10.8)
Genitourinary	7 (7.5)
Other[a]	4 (4.3)
Cancer stage N (%)	
Locoregional	31 (33.3)
Metastatic	62 (66.7)
Cancer treatment N (%)	
Chemotherapy[b]	31 (33.3)
Biologic agent[c]	13 (14.0)
Hormonal therapy[d]	3 (3.2)
Immunotherapy[e]	16 (17.2)
Chemotherapy + immunotherapy	4 (4.3)
Chemotherapy + biologic agent	17 (18.3)
Hormonal therapy + biologic agent	5 (5.4)
Radiotherapy	3 (3.2)
Radiotherapy + chemotherapy	1 (1.1)

[a] Other: brain, thymoma, endometrial, and neuroendocrine.
[b] Chemotherapy: Adriamycin, AC-T, AC-TPH, CMF, pemetrexed, cisplatin, carboplatin, capecitabine, paclitaxel, nab-paclitaxel, TDM-1, FOLFOX, FOLFIRI, FOLFIRINOX, gemcitabine, and vinorelbine.
[c] Biologic agents: bevacizumab, panitumumab, cetuximab, palbociclib, entrectinib, abemaciclib, trastuzumab, lenvatinib, neratinib, rucaparib, osimertinib, and dabrafenib.
[d] Hormonal therapy: letrozole, anastrazole, goserelin, megestrol, and octreotide.
[e] Immunotherapy: pembrolizumab, nivolumab, atezolizumab, cemiplimab, ipilimumab, and durvalumab.

neuroendocrine). The disease stage was local in 33.3% and metastatic in 66.7% of patients.

3.2. Immunogenicity six months following BNT162b2 vaccination

At a mean time of 176 days after the second vaccine dose, 78/93 (83.9%) patients with cancer developed RBD-IgG compared with 179/186 (96.3%) controls, p = 0.0001 (Table 3). The GMT of RBD-IgG was lower among patients with cancer than that of controls, 2.33 (95% CI 2.0−2.7) vs. 3.2 (95% CI 3.0−3.4), respectively, p = 0.0002. The GMT of Nabs was similar between patients with cancer and controls, 96.7 (95% CI 71.2−131.2) versus 87.5 (95% CI 74.6−102.7), respectively, p = 0.556 (Table 3).

Blood lymphocyte and neutrophil counts were recorded at three time points, the first at a median of ten days (5−17) before the first vaccine dose, the second at a median of 11 days (5−15) before the second vaccine dose, and the third at a median of 13.5 days (8−24) after the second vaccine dose. Neither lymphocyte nor neutrophil count were correlated with either RBD-IgG or Nabs titres at six months after the second vaccine dose (Supplementary Table 1).

Table 3
Antibody response and titres among patients with cancer and controls.

	Patients with cancer (N = 93)	Controls (N = 186)	p-value
RBD-IgG positive N (%)	78 (83.9)	179 (96.3)	0.0001
RBD-IgG titre GMT (95%CI)	2.3 (2.0–2.7)	3.2 (3.0–3.4)	0.0002
Neutralising Ab titre GMT (95%CI)	96.7 (71.3–131.2)	87.5 (74.6–102.7)	0.566

RBD, receptor-binding domain; IgG, immunoglobin G; CI, confidence interval; Ab, antibody; GMT, geometric mean.

4. Discussion

This is a prospective study evaluating serological responsiveness and neutralising antibodies levels in response to two vaccine doses mRNA BNT162b2 vaccine among patients with solid tumours receiving active treatment. We found that 84% of patients with cancer were seropositive six months following the second vaccine dose, compared with 96% of healthy controls. Median RBD-IgG titre was lower among patients compared with controls (2.3 versus 3.2). Among seropositive individuals, median Nabs titre was similar between patients with cancer and controls. Among patients with cancer, lymphocyte and neutrophil counts were not correlated with either RBD-IgG or Nabs titres at six months after the second vaccine dose.

A previous report showed that four months after the second vaccine dose, a lower percentage of patients with cancer were seropositive compared with healthy controls, 87% versus 100%, respectively [16]. Our findings support the notion that at six months after the second vaccine dose, the rate of seropositivity in patients with cancer is still lower than that of healthy controls. However, another study showed that at six months after vaccination, patients with cancer and healthy controls had similar seropositivity rates (79% versus 84%, respectively, p = 0.32) [17]. It should be noted that seropositivity rates of healthy controls shown by Waldhorn et al. were numerically lower than those shown in our study.

The efficacy of vaccines against SARS-CoV-2 is usually measured quantitively by RBD-IgG seropositivity rate and titre. Additionally, the functionality of the antibodies can be measured using neutralising antibodies assays [24]. Our findings show that compared with healthy controls, fewer patients with cancer mount an adequate immune response six months after vaccination. However, those patients with cancer that do achieve RBD IgG seropositivity, have a similar protection from SARS-CoV-2 infection, based on their level of Nabs.

Several studies attempted to define predictors for seronegativity following vaccination against SARS-CoV-2. In our previous report, only diabetes was associated with a lower rate of seropositivity in patients with cancer vaccinated with BNT162b2 [10]. Buttiron Webber et al. suggested that patients with cancer with baseline lymphocyte count below 1 x 10/L had a two-fold risk of seronegativity two weeks following vaccination with BNT162b2 [20]. Similarly, Sekkate et al. found a significant correlation between lymphocyte count and antibody level [21]. On the contrary, in the present study, we show that lymphocyte count at various time points before and after vaccination is not associated with seronegativity at six months after vaccination with BNT162b.

This study had several limitations. First, the data was collected during the time period in which the dominant SARS-CoV-2 variants were Alpha (until June 2021), and Delta thereafter. Second, we used humoral response as a surrogate for vaccine efficacy, yet we neither checked T cell activity against the virus nor showed clinical outcomes. Third, this study included a small sample size, and was conducted in a single centre.

5. Conclusions

This study demonstrated that seropositivity rates and RBD-IgG titre at six months after second BNT162b2 vaccine dose are lower among patients with cancer receiving anticancer therapy compared with healthy controls. However, Nabs titre is similar between these two groups, suggesting a comparable protection in seropositive individuals. Lymphocyte count, as well as neutrophil count, is not predictive of antibody response.

Author contributions

Conceptualization: OM and ESS. Data curation: OM, ESS, EGL, LO and GR. Project administration: ESS and GR. Writing – original draft: OM, ESS, EGL, LO and GR. Writing – review and editing: OM, ESS, AI, RB, SH, MJ, EGL, LO, GRY, YL and GR. Validation: OM, ESS, EGL, LO and GR. Investigation: OM and ESS. Resources: ESS and GR. Formal analysis: OM, ESS, EGL, LO and GR. Methodology: OM, ESS, EGL, LO and GR. Supervision: ESS and GR.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships

that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.03.013.

References

[1] Dai M, Liu D, Liu M, Zhou F, Li G, Chen Z, et al. Patients with cancer appear more vulnerable to SARS-CoV-2: a multicenter study during the COVID-19 outbreak. Cancer Discov 2020;10: 783–91.

[2] Giannakoulis VG, Papoutsi E, Siempos II. Effect of cancer on clinical outcomes of patients with COVID-19: a meta-analysis of patient data. JCO Glob Oncol 2020;6:799–808.

[3] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.

[4] Kuderer NM, Choueiri TK, Shah DP, Shyr Y, Rubinstein SM, Rivera DR, et al. Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study. Lancet 2020;395:1907–18.

[5] Lee LY, Cazier JB, Angelis V, Arnold R, Bisht V, Campton NA, et al. COVID-19 mortality in patients with cancer on chemotherapy or other anticancer treatments: a prospective cohort study. Lancet 2020;395:1919–26.

[6] Liang W, Guan W, Chen R, Wang W, Li J, Xu K, et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncol 2020;21:335–7.

[7] Tian J, Yuan X, Xiao J, Zhong Q, Yang C, Liu B, et al. Clinical characteristics and risk factors associated with COVID-19 disease severity in patients with cancer in Wuhan, China: a multicentre, retrospective, cohort study. Lancet Oncol 2020;21:893–903.

[8] Venkatesulu BP, Chandrasekar VT, Girdhar P, Advani P, Sharma A, Elumalai T, et al. A systematic review and meta-analysis of cancer patients affected by a novel coronavirus. JNCI Cancer Spectr 2021;5:pkaa102.

[9] Yang K, Sheng Y, Huang C, Jin Y, Xiong N, Jiang K, et al. Clinical characteristics, outcomes, and risk factors for mortality in patients with cancer and COVID-19 in Hubei, China: a multicentre, retrospective, cohort study. Lancet Oncol 2020;21:904–13.

[10] Shmueli ES, Itay A, Margalit O, Berger R, Halperin S, Jurkowicz M, et al. Efficacy and safety of BNT162b2 vaccination in patients with solid cancer receiving anticancer therapy - a single centre prospective study. Eur J Cancer 2021;157:124–31.

[11] Barriere J, Carles M, Audigier-Valette C, Re D, Adjtoutah Z, Seitz-Polski B, et al. Third dose of anti-SARS-CoV-2 vaccine for patients with cancer: should humoral responses be monitored? A position article. Eur J Cancer 2022;162:182–93.

[12] Di Noia V, Pimpinelli F, Renna D, Maccallini MT, Gariazzo L, Cosimati A, et al. Rapid decline of humoral response to two doses of BNT162b2 vaccine in patients with solid cancer after six months: the urgent need of the additional dose. Eur J Cancer 2022;165:169–73.

[13] Amatu A, Pani A, Patelli G, Gagliardi OM, Loparco M, Piscazzi D, et al. Impaired seroconversion after SARS-CoV-2 mRNA vaccines in patients with solid tumours receiving anticancer treatment. Eur J Cancer 2022;163:16–25.

[14] Goshen-Lago T, Waldhorn I, Holland R, Szwarcwort-Cohen M, Reiner-Benaim A, Shachor-Meyouhas Y, et al. Serologic status and toxic effects of the SARS-CoV-2 BNT162b2 vaccine in patients undergoing treatment for cancer. JAMA Oncol 2021.

[15] Massarweh A, Eliakim-Raz N, Stemmer A, Levy-Barda A, Yust-Katz S, Zer A, et al. Evaluation of seropositivity following BNT162b2 messenger RNA vaccination for SARS-CoV-2 in patients undergoing treatment for cancer. JAMA Oncol 2021;7: 1133–40.

[16] Eliakim-Raz N, Massarweh A, Stemmer A, Stemmer SM. Durability of response to SARS-CoV-2 BNT162b2 vaccination in patients on active anticancer treatment. JAMA Oncol 2021.

[17] Waldhorn I, Holland R, Goshen-Lago T, Shirman Y, Szwarcwort-Cohen M, Reiner-Benaim A, et al. Six month efficacy and toxicity profile of BNT162b2 vaccine in cancer patients with solid tumors. Cancer Discov 2021.

[18] Mair MJ, Berger JM, Mitterer M, Gansterer M, Bathke AC, Trutschnig W, et al. Third dose of SARS-CoV-2 vaccination in hemato-oncological patients and health care workers: immune responses and adverse events - a retrospective cohort study. Eur J Cancer 2022 Feb 7;165:184–94. https://doi.org/10.1016/-j.ejca.2022.01.019. [Online ahead of print].

[19] Debie Y, Vandamme T, Goossens ME, van Dam PA, Peeters M. Antibody titres before and after a third dose of the SARS-CoV-2 BNT162b2 vaccine in patients with cancer. Eur J Cancer 2022; 163:177–9.

[20] Buttiron Webber T, Provinciali N, Musso M, Ugolini M, Boitano M, Clavarezza M, et al. Predictors of poor seroconversion and adverse events to SARS-CoV-2 mRNA BNT162b2 vaccine in cancer patients on active treatment. Eur J Cancer 2021; 159:105–12.

[21] Sekkate S, Stoekle HC, Mabro M, Billard D, Kennel T, Vasse M, et al. Letter comments on: efficacy and safety of BNT162b2 vaccination in solid cancer patients receiving anti-cancer therapy - a single-centre prospective study: interaction between lymphopenia and anti-SARS-CoV-2 antibodies after two injections of the Pfizer-BioNTech vaccine in patients with cancers treated by chemotherapy or immunotherapy. Eur J Cancer 2022;160:282–4.

[22] Oved K, Olmer L, Shemer-Avni Y, Wolf T, Supino-Rosin L, Prajgrod G, et al. Multi-center nationwide comparison of seven serology assays reveals a SARS-CoV-2 non-responding seronegative subpopulation. E Clin Med 2020;29:100651.

[23] Zettl F, Meister TL, Vollmer T, Fischer B, Steinmann J, Krawczyk A, et al. Rapid quantification of SARS-CoV-2-neutralizing antibodies using propagation-defective vesicular stomatitis virus pseudotypes. Vaccines (Basel) 2020;8.

[24] Fendler A, Shepherd STC, Au L, Wilkinson KA, Wu M, Byrne F, et al. Adaptive immunity and neutralizing antibodies against SARS-CoV-2 variants of concern following vaccination in patients with cancer: the CAPTURE study. Nat Can 2021;2:1305–20.