Evaluating the Reciprocal Effects of COVID-19 Vaccines and Immunotherapy on Oncology Patients Receiving Immunotherapy

Arif Hakan ONDER[1], Kubra Demir ONDER[2], Yesim CEKIN[3], Banu OZTURK[1], Erdal KURTOGLU[4]

[1] Health Sciences University Antalya Training and Research Hospital, Department of Medical Oncology
[2] Health Sciences University Antalya Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology
[3] Health Sciences University Antalya Training and Research Hospital, Department of Microbiology
[4] Health Sciences University Antalya Training and Research Hospital, Department of Hematology

ABSTRACT

The administration of immunotherapy and coronavirus disease (COVID-19) vaccines can concurrently enhance systemic immune responses. Consequently, it is hypothesized that this potential overlapping immunological enhancement from the two treatments may result in an increased occurrence of immune-related adverse events. This study aimed to demonstrate the reciprocal effects of COVID-19 vaccines and immunotherapies. In this prospective study, the type and number of COVID-19 vaccines, levels of vaccine-induced antibodies, lymphocyte subtype counts, oncological treatments, response to immune therapy, adverse events, and involvement of lymph nodes (LN) were evaluated in cancer patients and healthy volunteers. Patients who received the BioNTech vaccine regimen had a higher rate of partial response to immune therapy at 3 months. There was no significant difference in the mean vaccine-induced antibody levels between the patient and control groups. In predicting mortality, pre- and post-vaccination CD20+ lymphocyte counts, post-vaccination C-reactive protein and lactate dehydrogenase levels, total lymphocyte count, albumin level, and LN size were significant ($p= 0.011$, $p< 0.001$, $p= 0.005$, $p= 0.003$, $p= 0.001$, $p< 0.001$, and $p= 0.001$, respectively). In this study, the relationship between peripheral blood T and B lymphocytes, immune responses, and adverse events were clearly demonstrated. Despite the majority of patients receiving inactivated vaccines as their first dose, the absence of significant differences in antibody levels between the patient and healthy volunteer groups highlights the influence of immunotherapy on the vaccine response. The group receiving the BioNTech vaccine exhibited better treatment response results at 3 months post-vaccination than the group without BioNTech. The types of adverse events displayed distinct changes in peripheral lymphocyte counts.

Keywords: COVID 19 vaccines, Immunotherapy, Lymphocyte counts

INTRODUCTION

The number of vaccine development studies on COVID-19 has rapidly increased in response to the global spread of the disease, resulting in the production of numerous vaccines with different mechanisms of action. The two most widely used vaccine types globally and in our country are the mRNA-based Pfizer BioNTech® and inactivated Sinovac (Coronavac)® vaccines.

Immune checkpoint inhibitors (ICI) have emerged as a significant advancement in oncology treatment. Compared with conventional chemotherapy, ICIs offer a more effective treatment option that can yield durable responses in specific patient populations[1] However, the distinction between ICIs and chemotherapy extends beyond their efficacies. ICIs also bring about a unique clinical profile of adverse events known as immune-related adverse events (irAEs). These adverse events are characterized by autoimmune manifestations that can affect any organ in the body following ICI administration. However, the underlying causes of irAEs are yet to be fully elucidated.[2]

Theoretically, administration of both immunotherapy and COVID-19 vaccines can concurrently enhance systemic immune responses.

Consequently, it is hypothesized that this potential overlapping immunological enhancement from the two treatments may result in an increased occurrence of irAEs. Although irAEs in patients receiving ICIs are typically mild to moderate in severity, they can occasionally be severe or even fatal.[3]

Recent studies have confirmed the safety and efficacy of COVID-19 vaccination in cancer patients undergoing immunotherapy. Mei et al. demonstrated in a real-world study that COVID-19 vaccination does not impair the use of PD-1 inhibitors in cancer treatment, showing continued treatment efficacy without increased adverse events.[4] Similarly, Ruiz et al. conducted a systematic review and meta-analysis, which further supports the safety of COVID-19 vaccines in patients receiving immune checkpoint inhibitors, with no significant increase in immune-related adverse events.[5] These findings provide robust evidence that COVID-19 vaccines can be safely administered to cancer patients receiving immunotherapy, reinforcing their recommended use during treatment. In cancer patients receiving immunotherapy, ICIs induce immune-related adverse events in approximately 20-50% of cases, with a higher risk observed in elderly patients.[6,7]

Accordingly, concerns have arisen regarding the administration of both mRNA COVID-19 vaccines and immunotherapy in this patient population due to the potential for excessive immune system activation. Concurrent administration of vaccines and immunotherapy has the potential to potentiate each other's activities, leading to mutually reinforcing effects.[8]

Several studies focusing on vaccines have demonstrated that immune checkpoint inhibitor (ICI) treatments such as atezolizumab, nivolumab, and pembrolizumab do not significantly compromise vaccine efficacy. Thus, COVID-19 vaccines are expected to elicit an appropriate immune response in patients undergoing ICI treatment. However, it is important to note that vaccination of ICI-treated patients may increase the risk of irAEs and potentially lead to a cytokine storm due to immune system overstimulation.[9] Although the BNT162b2 mRNA vaccine has been reported to increase the occurrence of adverse events, short-term safety data for the vaccine are available.[10] Notably, studies in the literature have indicated that influenza vaccination improves the survival of patients receiving ICI therapy without causing any adverse effects.[11,12]

However, it should be acknowledged that COVID-19 vaccines generally exhibit more significant adverse events than influenza vaccines do. Therefore, the impact of COVID-19 vaccines on ICI-related adverse events should not be overlooked.[8] In light of these considerations, this study aimed to investigate the effect of immune therapy on the COVID-19 vaccine antibody response in cancer patients receiving ICI treatment, and to determine whether these vaccines have any effect on immunotherapy treatment response and adverse events.

PATIENTS AND METHODS

This study was designed as a prospective, non-interventional, observational study with a control group. This study did not employ randomization as it was designed as a real-world observational study. However, patient selection criteria were clearly defined to ensure consistency and minimize bias. Inclusion criteria comprised cancer patients receiving immune checkpoint inhibitors (ICIs) with no active COVID-19 infection at the time of enrollment. Exclusion criteria included patients requiring systemic immunosuppression or those with a recent history of COVID-19 infection. The control group consisted of healthy volunteers matched by age and gender with the patient group. We acknowledge the difference in follow-up durations between the groups as a limitation caused by logistical challenges during the pandemic.

The study included patients aged 18 years or older with solid organ cancers who were receiving immunotherapy, as well as healthy volunteers. Patients were required to have no history of COVID-19. The study did not intervene in the patients' decisions regarding COVID-19 vaccination or the choice of vaccine. The patients were followed up for 18 months, while the healthy volunteer group was followed up for 6 months.

Blood samples were collected from both healthy volunteers and patients before receiving the COVID-19 vaccine and after receiving at least one dose

of the vaccine. The samples were used to measure SARS-CoV-2 antibody levels and CD4, CD8, CD19, and CD20+ lymphocyte counts using flow cytometry. In addition, markers of inflammation such as neutrophils, lymphocytes, platelets, albumin, C-reactive protein (CRP), lactate dehydrogenase (LDH), and calcium levels were measured before vaccination and after two doses of the vaccine. Tumor size was assessed using tomography and the diameter of the largest lymph node was determined using positron emission tomography (PET-CT). Furthermore, comparisons were made between the maximum standardized uptake values (SUVmax) obtained from PET/CT scans. The control group was followed for a period of 6 months, compared to the 12-month follow-up of the patient group. This difference was due to logistical challenges during the pandemic, particularly regarding the availability of healthy volunteers for prolonged follow-up. While this is acknowledged as a limitation, it is unlikely to have significantly influenced our results, as key immune responses were observed within the first few months following vaccination.

Demographic data of the patients, including the types of immunotherapy drugs they received, type of COVID-19 vaccine administered, number of vaccine doses, and whether the patients or healthy volunteers were diagnosed with COVID-19, were also recorded.

Flow Cytometry

Flow cytometry is a technique used to measure and analyze cells at the individual cell level, based on their size, granularity, and fluorescence intensity. The cells in the liquid suspension were propelled through a flow chamber using air pressure. The high hydrostatic pressure created by the fast flow of the liquid pushes the cells into the flow chamber, which is typically made of glass or quartz (flow gel), where their characteristics are measured.

Evaluation of Immunotherapy Response The response to immunotherapy was evaluated as follows:

Tumor size reduction: This involves comparing the size of the tumor before and after vaccination to assess the degree of reduction.

Comparison of PET/CT SUVmax values: The SUVmax obtained from PET/CT scans was used to compare the metabolic activity of the tumor before and after vaccination.

Comparison of lymphocyte counts: The counts of specific lymphocyte subsets, such as CD4, CD8, CD19, and CD20+, were measured before and after vaccination to evaluate any changes.

Measurement of reactive lymph nodes: PET-CT scans are used to detect and measure the presence of reactive lymph nodes after vaccination.

Based on the treatment responses observed, patients were categorized into different groups: partial response (PR, Reduction of at least 30% in tumor burden); Complete response (CR, The complete disappearance of lesions and lymph node diameters less than 10 mm); Progressive disease (PD, The worsening of the disease with an increase of ≥ 20 in tumor burden and an absolute minimum increase of 5 mm compared to nadir or the emergence of new lesions); and stable disease (SD; a disease state that does not meet the criteria for PR, CR, or PD).

Effect of the COVID-19 Vaccine on Immunotherapy Efficacy and Adverse Effects

a) Immunotherapy responses according to the type of vaccine administered were assessed at 3, 6, and 12 months.

b) Immunotherapy responses by the number of vaccines received were evaluated.

c) Whether the COVID-19 vaccine affected the occurrence of adverse events associated with immunotherapy was examined.

- Investigation of the effect of vaccine type on immunotherapy-related AEs
- Investigation of the effect of the number of vaccines on immunotherapy-related AEs
- Investigation of the effect of post-vaccination antibody levels on immunotherapy related AEs

Effect of Immunotherapy on Vaccine-induced Antibody Levels

The study population was divided into two groups: oncology patients who received immunotherapy

and healthy volunteers. Antibody levels in response to the vaccine were assessed in both groups.

Microbiological Technique

The antibody levels in our study were measured using the Elecsys Anti-SARS-CoV-2 S immunoassay, a commercial kit that utilizes the electrochemiluminescence (ECLIA) method. This kit specifically detects high-affinity antibodies against the receptor-binding site (RBD) of the SARS-CoV-2 S protein. The measurements were performed on human serum samples using Cobas immunoassay analyzers following the manufacturer's guidelines (Roche Diagnostics). The analyte concentration in each sample was automatically reported in units of U/mL. Results below 0.80 U/mL were considered negative for anti-SARS-CoV-2 S antibodies.

Ethical approval: This study was approved by the Institutional Review Board of the Antalya Education and Research Hospital Ethics Committee for Clinical Trials (Approval No: 14/1- September 16, 2021).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA). Descriptive statistics were reported as counts and percentages for categorical variables, and as mean ± standard deviation or median (interquartile range) for continuous variables. Normality assumptions were assessed using the Kolmogorov-Smirnov test. For comparisons between continuous variables and groups, parametric tests such as ANOVA and nonparametric tests such as the Kruskal-Wallis test were employed based on the normality of the data. Spearman correlation test, a nonparametric test, was used to determine the relationship between continuous variables. The independent t-test, parametric test, and– the Whitney U test, a non-parametric test, were conducted to identify significant differences between the patient and control groups. Categorical variables were compared using the chi-squared test or Fisher's exact test. The results of the ROC analysis, which predicted mortality based on various variables, are presented. Survival times and progression-free survival times were compared using the Kaplan-Meier method. Statistical significance was set at $p< 0.05$.

RESULTS

The study included 32 patients who received immunotherapy between March 1, 2021, and June 31, 2021. The control group consisted of 19 healthy volunteers recruited between June 1, 2021, and December 31, 2021. Of these patients, 29 (90.6%) were male. The mean age of the patient group was 60.75±10.08 years, with a median age of 64. The healthy volunteer group had a mean age of 8±7.6 years, with a median age of 45. In terms of cancer types, 12 patients (37.5%) had lung cancer, with 9 of them diagnosed with Non-Small Cell Lung Cancer (NSCLC). Malignant melanoma was observed in 10 patients (31.3%), renal cell carcinoma in 9 patients (28.1%), and hepatocellular carcinoma in 1 patient (3.1%). At the time of diagnosis, 15 patients (46.9%) had advanced stage (stage 4) cancer. Lymph node metastases were detected in 87.5% of patients, while lung metastases were observed in 62.5% of patients. The most commonly used treatment was nivolumab (n= 25.1%). The mean number of immunotherapy cycles received by the patients was 14.19±16.1, with a median of eigth. Twenty-two patients (68.8%) had a performance score of 1. Table 1 provides detailed demographic and clinical information on the patient group.

Analysis of the patients regarding COVID-19 vaccine doses and types revealed that patients (96.9%) received at least two doses of the vaccine during the follow-up period. Among them, 18 patients (56.25%) received three or more doses. The first dose of the vaccine was Sinovac (CoronaVac)® in 68.8% of patients, while the second dose was Sinovac (CoronaVac)® in 53.1% of patients. For the third dose, 84.4% of patients received Pfizer-BioNTech®. In total, 26 patients (81.25%) received at least one dose of Pfizer-BioNTech® vaccine. None of the patients had a history of COVID-19 infection. However, when measuring pre-vaccination antibody levels, it was found that four patients had a total antibody level > 250 U/mL, three patients had low antibody levels, and 25 patients had nega-

Table 1. Demographic and clinical information about patients (n= 32)

Variables	n	%
Age (Mean±SD, Median) (year)	60.75±10.08	64.00
Gender		
Male	29	90.6
Female	3	9.4
Cancer type		
LC (SCLC)	3	9.4
LC (NSCLC)	9	28.1
HCC	1	3.1
Malignant melanoma	10	31.3
RCC	9	28.1
Immune therapy received		
Atezolizumab	6	18.8
Ipilimumab-Nivolumab	1	3.1
Nivolumab	25	70.1
Pre-immunotherapy treatments		
Never received treatment	2	6.3
Tyrosine Kinase Inhibitor (TKI)	12	37.5
Chemotherapy	18	56.3
Number of chemotherapy and TKI received before immunotherapy		
0	2	6.3
1	22	68.8
2	8	25.0
Receiving chemotherapy with immunotherapy		
0	29	90.6
1	3	9.4
Cancer stage at diagnosis		
2	4	12.5
3	13	40.6
4	15	46.9
Lung metastasis		
No	12	37.5
Yes	20	62.5
Liver metastasis		
No	27	84.4
Yes	5	15.6
Bone metastasis		
No	23	71.9
Yes	9	28.1
Lymph node metastasis		
No	4	12.5
Yes	28	87.5
Brain metastasis		
No	27	84.4
Yes	5	15.6
Adrenal metastasis		
No	28	87.5
Yes	4	12.5
Other metastasis		
No	31	96.9
Yes	1	3.1
Performance score		
1	22	68.8
2	10	31.3

LC: lung cancer; SCLC: Small cell lung cancer; NSCLC: Non-small cell lung cancer; HCC: Hepatocellular carcinoma; RCC: Renal cell carcinoma; TKI: Tyrosine kinase inhibitor

tive antibody levels. Of the healthy volunteers, 15 (78.9%) received three doses or more of the vaccine. None of the healthy volunteers had a history of COVID-19. Prior to vaccination, 18 healthy volunteers had negative antibody levels, whereas only one individual had a level of 28 U/mL. Table 2 provides detailed information on the COVID-19 vaccine type, dose, and antibody levels before and after vaccination in patients and healthy volunteers.

Analysis of immunotherapy-associated AEs following administration of COVID-19 vaccine is shown in Table 3. Table 3 presents a detailed distribution of immunotherapy-related AEs vaccine showed that 25 (78%) of the 32 patients experienced AEs, with the most common AE being thyroid dysfunction, which affected 52% of the patients. Of the 13 patients who experienced thyroid dysfunction, five (40.6%) had grade III AEs, three had grade II AEs, and nine had grade I AEs. Colitis, the second most frequent AE, was observed in 9 patients (36%). Of these cases, one was classified as grade III, five as grade II, and three as grade I. Dermatitis, the third most prevalent AE, was reported in eight (32%) patients. One case was categorized as grade III, three as grade II, and four as grade I.

The study also identified a few rare AEs, including hepatitis in two patients (both grade II), activation of autoimmune disease in two patients (one grade II and one grade I), and carditis in one patient (grade II). Overall, of the total 35 AEs, 3 (8.5%) were classified as grade III, 15 (42.8%) as grade II, and 17 (48.5%) as grade I. Table 3 presents a detailed distribution of immunotherapy-related AEs following COVID-19 vaccination. CD4, CD8, CD19, and CD20+ lymphocyte counts were analyzed according to pre- and post-vaccination immunotherapy-related AEs in the patients receiving immunotherapy. When comparing post-vaccination changes to pre-vaccination levels, it was observed that individuals without AEs had decreased levels of CD4, CD8, CD19, and CD20+ lymphocytes. Conversely, the highest increase in lymphocyte count was found in CD19+ lymphocytes among those with AEs, such as thyroiditis, thyroiditis+colitis, colitis, and dermatitis. In cases of thyroiditis+activation of underlying autoimmune disease, the CD4+ lymphocyte count showed

Table 2. COVID-19 vaccine and antibody information about patients and healthy volunteers

Variables of patients (n= 32)	n	%
Number of vaccine doses during the follow-up period		
1	1	3.1
2	13	40.6
3	6	18.8
4	6	18.8
5	4	12.5
6	2	6.3
Pfizer-BioNTech®		
None	6	18.8
1 dose	5	15.6
2 doses	15	46.9
3 doses	4	12.5
4 doses	2	6.3
Sinovac (CoronaVac)®		
None	8	25.0
1 dose	8	25.0
2 doses	12	37.5
3 doses	1	3.1
4 doses	2	6.3
5 doses	1	3.1
Vaccine type at first dose		
Pfizer-BioNTech®	10	31.3
Sinovac (CoronaVac)®	22	68.8
Vaccine type at second dose		
Pfizer-BioNTech®	15	46.9
Sinovac (CoronaVac)®	16	53.1
Vaccine type at third dose		
Pfizer-BioNTech®	13	84.4
Sinovac (CoronaVac)®	5	15.6
Pre-vaccination antibody level (U/mL)		
> 250	4	12.5
11	1	3.1
23	1	3.1
52	1	3.1
Neg	25	78.1
Post-vaccination antibody level (U/mL)		
> 250	20	62.5
231	1	3.1
199	1	3.1
198	1	3.1
194	1	3.1
176	1	3.1
131	1	3.1
39	1	3.1
38	1	3.1
37	1	3.1
22	1	3.1
19	1	3.1
1,4	1	3.1
Number of BioNTech® doses before antibody measurement		
0	15	46.9
1	9	28.1
2	8	25.0
Number of Sinovac® doses before antibody measurement		
0	10	31.3
1	13	40.6
2	7	21.9
3	2	6.3

To be continued

Table 2. ***(Continue)***

Variables of patients (n= 32)	n	%
Total number of vaccine doses before antibody measurement		
1	13	40.6
2	13	40.6
3	5	15.6
4	1	1
Variables of healthy volunteers (n=19)	**n**	**%**
Number of vaccine doses during the follow-up period		
1	0	0.0
2	4	21.1
3	11	57.8
4	3	15.8
5	1	5.3
6	0	0.0
BioNTech®		
0	1	5.3
1	1	5.3
2	4	21.1
3	11	57.9
4	2	10.5
Sinovac (CoronaVac)®		
0	15	78.9
1	0	0.0
2	4	21.1
3	0	0.0
4	0	0.0
5	0	0.0
Vaccine type at first dose		
Pfizer-BioNTech®	15	78.9
Sinovac (CoronaVac)®	4	21.1
Vaccine type at second dose		
Pfizer-BioNTech®	15	78.9
Sinovac (CoronaVac)®	4	21.1
Vaccine type at third dose		
Pfizer-BioNTech®	15	78.9
Sinovac (CoronaVac)®	0	0.0
Turkovac®	4	21.1
Pre-vaccination antibody level (U/mL)		
28	1	5.3
Negative	18	94.7
Post-vaccination antibody level (U/mL)		
> 250	15	78.9
64	1	5.3
34	1	5.3
17	1	5.3
11	1	5.3
Number of BioNTech® doses before antibody measurement		
0	3	15.8
1	0	0.0
2	16	84.2
Number of Sinovac® doses before antibody measurement		
0	16	84.3
1	1	5.3
2	2	10.5
3	0	0.0
Total number of vaccine doses before antibody measurement		
1	1	5.3
2	18	94.7
3	0	0.0
4	0	0.0
Vaccine type		
Vaccine regimen without BioNTech®	3	15.8
Vaccine regimen with BioNTech®	16	84.2

Table 3. Distribution of immunotherapy-related adverse events following COVID-19 vaccines (n= 32)

Adverse event	n	%
None	7	21.9
Thyroid dysfunction	7	21.9
Thyroid dysfunction and colitis	2	6.3
Thyroid dysfunction and hepatitis	1	3.1
Thyroid dysfunction and activation of underlying autoimmune disease	2	6.3
Thyroid dysfunction and carditis	1	3.1
Colitis	4	12.5
Colitis and dermatitis	3	9.4
Dermatitis	4	12.5
Dermatitis and hepatitis	1	3.1

the highest increase, while other lymphocyte subsets demonstrated a decrease. The CD8+ lymphocyte count showed the highest increase in individuals with colitis and dermatitis. Rare AEs were also identified, including thyroiditis+hepatitis in one patient, thyroiditis+carditis in another patient, and dermatitis+hepatitis in one patient. In cases of post-vaccination thyroiditis+carditis, all lymphocyte subsets, particularly CD19+ B lymphocytes (126/mm^3 (64%), decreased. Conversely, in cases of thyroiditis+hepatitis, only CD8+ lymphocytes (144/mm^3 (26%) increased, while the others decreased. In cases of dermatitis+hepatitis, CD19 (160/mm^3 (85%)) and CD8+ lymphocytes (140/mm^3 (81%) increased, while CD20+ lymphocytes (12/mm^3 (33%) decreased. Table 4 provides an overview of the changes in CD4, CD8, CD19, and CD20+lymphocyte counts and levels before and after vaccination categorized by AEs.

Spearman correlation analysis to examine the relationship between the number of involved lymph node (LN) regions and post-vaccination antibody levels in patients with reactive or ametabolic LN involvement after the first two doses of vaccination showed no significant correlation between these variables (r= –0.082, p= 0.656). Further analysis to explore the relationship between the number of involved lymph node regions and the differences in CD4, CD8, CD19, and CD20+ lymphocyte counts before and after vaccination in patients with reactive or ametabolic LN involvement revealed statistically significant positive correlations between the number of involved lymph node regions and differences in CD19 (r= 0.359, p= 0.044) and CD20 (r= 0.465, p= 0.007).

Additionally, a positive and statistically significant correlation was observed between the increase in lymph node size (in mm) and the difference in CD20+ lymphocyte count in patients with reactive or ametabolic LN involvement after the first two doses of vaccination (r= 0.415, p= 0.044).

Furthermore, a positive correlation was found between the presence of reactive or ametabolic LN involvement and the number of involved LN regions as well as the LDH change after the first two doses of vaccination (r= 0.362, p= 0.042).

Reactive lymph nodes were primarily detected in the axillary and cervical regions during follow-up imaging. However, no significant relationship was observed between the location of the reactive lymph nodes and the site of vaccine administration. These findings suggest that the lymph node enlargement is more likely due to a generalized immune response rather than a localized reaction to the vaccine. The comparison of "lymph node involvement and the number of involved regions" with immune treatment responses at 3 months, 6 months, and 1 year, and AE status after two doses of vaccination in the patient group did not reveal any significant relationship (Table 5).

The immune treatment responses in the patient group were assessed at the end of the third, sixth, and first years after vaccination. The results revealed a gradual increase in the partial response rate following vaccination, with rates of 12.5%, 17.9%, and 33.3% at 3 months, 6 months, and 1 year, respectively. Conversely, the rate of progressive disease was higher at 6 months (35.7%) than at 3 months (25%), while the lowest rate was observed during the 1-year follow-up for the immune response (16.7%).

The relationship between the type of vaccine and the immune therapy response was examined. The results indicated no significant relationship between the vaccine regimens with and without BioNTech and the 6-month and 1-year immunotherapy responses. However, the proportion of patients with a partial response to immune therapy at

Table 4. Changes in CD 4-8-19-20+ lymphocyte counts and levels before and after vaccination by adverse events

Pre-vaccination CD 4-8-19-20 levels (cells/mm³) by adverse events (mean±SD)				
Adverse event	**CD 4**	**CD 8**	**CD 19**	**CD 20**
None	541.85±314.82	475.42±305.62	576.71±446.78	129.00±136.78
Thyroiditis	606.57±308.37	551.28±183.08	606.71±184.41	103.85±76.07
Thyroiditis and colitis	531.50±94.04	653.50±181.72	730.00±268.70	114.00±33.94
Thyroiditis and activation of underlying autoimmune disease	647.00±103.23	304.40±124.45	428.00±147.07	131.00±32.52
Colitis	406.00±100.54	356.25±73.21	428.50±105.13	92.25±64.77
Colitis and dermatitis	750.66±154.27	407.33±98.23	564.66±99.20	176.66±37.54
Dermatitis	509.25±469.25	518.00±212.62	601.75±244.58	152.75±144.29
Rare adverse events	**Pre-vaccination/mm³**			
	CD4	**CD 8**	**CD19**	**CD20**
Thyroiditis and hepatitis (n=1)	688	560	640	112
Thyroiditis and carditis (n=1)	180	126	198	18
Dermatitis and hepatitis (n=1)	120	172	188	36
Post-vaccination CD 4-8-19-20 levels by adverse events (cells/mm³) (mean±SD)				
Adverse event	**CD 4**	**CD 8**	**CD 19**	**CD 20**
None	449.12±325.78	360.85±263.79	503.71±369.79	101.42±138.62
Thyroiditis	388.52±359.95	760.71±566.66	819.57±538.52	115.00±97.06
Thyroiditis and colitis	564.00±73.53	836.00±322.44	960.00±543.05	204.00±107.48
Thyroiditis and activation of underlying autoimmune disease	749.00±606.69	198.50±202.93	300.00±247.48	111.00±57.98
Colitis	511.80±368.10	567.60±276.99	700.70±442.92	160.35±136.12
Colitis and dermatitis	890.33±127.81	654.00±83.59	736.00±132.18	161.66±59.60
Dermatitis	781.00±479.12	766.00±464.47	1031.00±580.06	276.00±192.02
Rare adverse events	**Post-vaccination/mm³**			
	CD4	**CD 8**	**CD19**	**CD20**
Thyroiditis and hepatitis (n=1)	640	704	560	144
Thyroiditis and carditis (n=1)	132	56	72	16
Dermatitis and hepatitis (n=1)	156	312	348	24
Changes in CD 4-8-19-20 levels before and after vaccination by adverse events (mean±SD)				
Adverse event	**CD 4**	**CD 8**	**CD 19**	**CD 20**
None	-92.71±126.29	-114.57±167.53	-73.00±207.28	-27.57±35.19
Thyroiditis	114.85±162.12	209.42±422.44	212.85±421.04	11.14±24.84
Thyroiditis and colitis	32.50±20.50	182.50±140.71	230.00±274.35	90.00±73.53
Thyroiditis and activation of underlying autoimmune disease	102.00±503.46	-105.5±327.39	-128.00±394.56	-20.00±90.50
Colitis	105.80±319.99	211.35±287.07	272.20±383.33	66.10±79.95
Colitis and dermatitis	139.66±127.11	246.66±28.44	171.33±33.00	-15.00±55.65
Dermatitis	271.75±221.62	248.00±400.09	429.25±379.35	123.25±101.71
Rare adverse events	**Change (Δ)/mm³ (%)**			
	CD4	**CD 8**	**CD19**	**CD20**
Thyroiditis and hepatitis (n=1)	-48 (7%)	144 (26%)	-80 (13%)	32 (29%)
Thyroiditis and carditis (n=1)	-48 (27%)	-70 (56%)	-126 (64%)	-2 (11%)
Dermatitis and hepatitis (n=1)	36 (30%)	140 (81%)	160 (85%)	-12 (33%)

3 months was higher among those who received a vaccine regimen with BioNTech (p= 0.046).

Patients were assessed based on whether they developed irAEs following vaccination, and their immune therapy response was examined. Although there was no significant difference in the treatment responses at the 3-month and 6-month post-vaccination follow-ups based on AE status, it was observed that the 1-year immune therapy response was better in patients who experienced AEs, with this difference being statistically significant (p=

Table 5. Comparison of lymph node involvement and the number of involved regions with immune treatment responses at 3 months, 6 months and 1 year, and AE status after two doses of vaccination in the patient group

LN involvement and number of involved LN regions and adverse event status after the first two doses of vaccination	**0** (n= 8)	**1** (n= 16)	**2** (n= 7)	**3** (n= 1)	**p-value**
Post-vaccination 3-month immune therapy response					
CR	0 (0)	2 (100)	0 (0)	0 (0)	0.647
PR	1 (25)	1 (25)	2 (50)	0 (0)	
Stable	5 (27.8)	10 (55.6)	2 (11.1)	1 (5.6)	
PD	2 (25)	3 (37.5)	3 (37.5)	0 (0)	
Post-vaccination 6-month immune therapy response					
PR	0 (0)	2 (40)	2 (40)	1 (20)	0.506
Stabil	2 (15.4)	8 (61.5)	3 (23.1)	0 (0)	
PD	3 (30.0)	5 (50.0)	2 (20.0)	0 (0)	
Post-vaccination 1-year immune therapy response					
PR	0 (0)	2 (33.3)	3 (50)	1 (16.7)	0.361
Stable	0 (0)	6 (66.7)	3 (33.3)	0 (0)	
PD	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)	
Adverse event					
No	3 (50.0)	3 (50.0)	0 (0.0)	0 (0.0)	0.359
Yes	5 (19.2)	13 (50.0)	7 (26.9)	1 (3.8)	

Fisher's Exact Test; CR= complete response; PR= partial response; PD= progressive disease

0.020). Of the 18 patients who completed the 1-year follow-up after vaccination, all six patients with partial response (PR) and all nine patients with stable disease were in the AE group. Among the three patients with progressive disease (PD), two were in the non-AE group and one was in the AE group. The relationship between post-vaccination CD4, CD8, CD19, and CD20+ lymphocyte counts and CRP levels was examined in the patient group. The results revealed a statistically significant negative correlation between CD4+ and CD20+ lymphocyte levels and CRP level (r= –0.441; p= 0.001 and r=–0.452; p= 0.001, respectively).

Changes in pre- and post-vaccination laboratory parameters were also evaluated in the patient group based on the immune therapy response within the first three months after vaccination. It was observed that the rate of "progressive disease" was higher among those whose neutrophil levels (cells/mm3) decreased after vaccination compared to pre-vaccination values, while the rate of "stable disease" was higher among those whose neutrophil levels increased after vaccination compared to pre-vaccination values, and this difference was statistically significant (-1610±2940 vs. 1550±2310, respectively; p= 0.022). However, no significant relationship was found between the 6-month immune therapy responses and laboratory parameters. Furthermore, when the relationship between first-year immune therapy responses and laboratory parameters was examined, it was noted that the "partial response rate" was higher in patients whose albumin levels (g/L) increased after vaccination. In contrast, patients with "stable or progressive disease" exhibited a decrease in albumin levels after vaccination, with this difference being statistically significant (4.17±5.00 and -3.33±5.43 and –1.33±2.89, respectively; p= 0.039).

This study evaluated the relationship between target lesion diameters, SUVmax values, and peripheral blood CD4, CD8, CD19, and CD20+ lymphocyte levels during the post-vaccination follow-up period. The results indicated a statistically significant negative correlation between the sum of post-vaccination 3-month target lesion diameters (mm) and the change in CD8+ lymphocyte count (r= –0.366, p= 0.040) and CD20+ lymphocyte count (r= –0.428, p= 0.014).

Furthermore, a significant negative correlation was observed between the post-vaccination 3-month SUVmax value of thetarget lesion and the change in CD8+ lymphocyte count (r=–0.504, p= 0.005).

The diameters of the target lesions and SUVmax values were examined 6 months post- vaccination. A negative correlation was found between the total number of immunotherapy sessions received until six months post-vaccination and the diameters of the target lesions (r=–0.417, p= 0.027). Similarly, a negative correlation was observed between the post-vaccination 6-month SUVmax values of the target lesions and the total number of immunotherapy sessions received until 6 months post-vaccination (r=–0.495, p= 0.010). Additionally, a negative correlation was found between the post-vaccination 6-month diameters of the target lesions and the change in the CD20+ lymphocyte count (r=–0.391, p= 0.040). However, no statistically significant correlation was observed between the post-vaccination 1-year diameters and SUVmax values of the target lesions and thevariables assessed (p> 0.05).

Among healthy volunteers, 12 (63.1%) developed COVID-19 during the postvaccination follow-up period. No significant differences were found in terms of vaccine type and number of vaccine doses among those who contracted COVID-19. Furthermore, there was no difference in post-vaccination antibody levels between healthy volunteers with and without COVID-19 (median 255 U/mL for both groups; p= 0.473). Additionally, no significant differences were observed in CD4, CD8, CD19, and CD20+ lymphocyte counts between healthy volunteers who had contracted COVID-19 and those who did not.

Comparison of Patient and Control (Healthy Volunteers) Groups

The number of male patients was significantly higher in the patient group (n= 29, 76.3%) than in the control group (n= 9, 23.7%, p= 0.002). Statistically significant differences were observed between the patient and healthy volunteer groups in terms of the number of BioNTech doses before antibody level measurement for the first and second doses (p< 0.001), number of Sinovac doses before antibody level measurement for the first dose (p= 0.002), number of total vaccine doses before antibody level measurement for both the first and second doses (p< 0.001), and vaccine type (p= 0.025). Despite these differences, there was no significant difference in mean antibody levels between the two groups (201.3 ± 90.07 U/mL vs. 207.9 ± 94.11 U/mL; p= 0.508). It was determined that 70.6% of those with COVID-19 were in the healthy volunteer group (p< 0.001). Pre-vaccination neutrophil and CRP levels were higher in the patient group than in the healthy volunteer group (p= 0.038 and p< 0.001, respectively). Conversely, pre-vaccination lymphocyte, albumin, and calcium levels were higher in the healthy volunteer group than in the patient group (p< 0.001, p< 0.001, and p= 0.002, respectively).

Post-vaccination CRP and LDH levels were higher in the patient group than in the healthy volunteer group (p< 0.001 vs. p= 0.007, respectively). Post-vaccination lymphocyte, albumin, and calcium levels were higher in the healthy volunteer group than in the patient group (p= 0.010, p= 0.012, p= 0.024, respectively).

Pre-vaccination CD4, CD8, CD19, and CD20+ lymphocyte counts and CD4/CD8 ratio were higher in the healthy volunteer group than in the patient group (p< 0.001, p= 0.009, p= 0.004, p= 0.003, p= 0.05, respectively). After vaccination, only CD4 and CD20+ lymphocyte counts were higher in the healthy volunteer group than in the patient group (p= 0.001 and p= 0.018, respectively). There were no significant differences between the patient and healthy volunteer groups in terms of changes in CD4, CD8, CD19, and CD20+ lymphocyte counts before and after vaccination. Although no statistically significant difference was found in the patient group for these lymphocytes, the post-vaccination change was higher than that in the healthy volunteer group.

To identify predictive factors for mortality, various variables, including pre- and post- vaccination routine laboratory parameters, CD4, CD8, CD19, and CD20+ lymphocyte counts, and reactive lymph node size observed during post-vaccination treatment response evaluation, were examined. The results revealed several significant predictors of mortality. These included the pre- and post-vaccination

CD20+ lymphocyte count, post-vaccine CRP level, post-vaccination LDH level, post-vaccination total lymphocyte count, post-vaccination albumin level, and post-vaccination LN size (p= 0.011, p< 0.001, p= 0.005, p= 0.003, p= 0.001, p< 0.001, and p= 0.001, respectively).

ROC analysis designed to determine the predictive value of pre-vaccination CD-20+ lymphocyte count for mortality showed an area under the curve (AUC) value of 0.738 (95% CI: 0.567-909). Using a cutoff value of pre-vaccination CD20+ lymphocyte count ≤ 104.5 (cells/ mm^3), the sensitivity for predicting mortality was 69.2%, with a selectivity of 68.4%. In the ROC analysis conducted to determine the predictive value of post-vaccination CD20+ lymphocyte counts for mortality, the AUC was 0.851 (95% CI: 0.708-0.994). A cut-off value of post-vaccination CD20+ lymphocyte count ≤94.5 (cells/ mm^3) had a sensitivity of 84.6% and selectivity of 6% for predicting mortality.

For the post-vaccination CRP level, the AUC in the ROC analysis was 0.761 (95% CI: 0.604-918). Using a cutoff value of post-vaccination CRP ≥ 11.5 mg/L, the sensitivity for predicting mortality was 69.2%, with a selectivity of 68.4%.

The ROC analysis performed to determine the predictive value of post-vaccination LDH level for mortality yielded an AUC of 0.778 (95% CI: 0.614-0.943). A cut-off value of post-vaccination LDH ≥ 211 U/L had a sensitivity of 69.2% and selectivity of 71.1% for predicting mortality.

In the ROC analysis conducted to determine the predictive value of post-vaccination lymphocyte counts for mortality, the AUC was 0.813 (95% CI: 0.644-0.982). Using a cutoff value of post-vaccination lymphocyte count ≤ 1650 (cells/mm^3), the sensitivity for predicting mortality was 9%, with a selectivity of 78.9%.

The AUC in the ROC analysis designed to determine the predictive value of the post-vaccination albumin level for mortality was 0.907 (95% CI: 0.793-1.000). A cutoff value of post-vaccination albumin ≤ 40.5 g/L had a sensitivity of 92.3% and a selectivity of 78.9% for predicting mortality.

Finally, the AUC in the ROC analysis performed to determine the predictive value of post-vaccination LN size for mortality was 0.981 (95% CI: 0.936-1.000). Using a cutoff value of post-vaccination LN size ≤ 8.5 mm, the sensitivity for predicting mortality was found to be 83.5%, with a selectivity of 94.4%.

The relationships between statistically significant parameters for predicting mortality and overall survival (OS) and progression-free survival (PFS) were examined. Accordingly, the median OS was determined to be 88.03 months (95% CI: 31.74-144.32). The 2-year survival rate were 79.5%, while the 5-year survival rate was 51.6%, respectively.

Regarding the median OS (months) by pre-vaccination CD20+ lymphocyte level, no statistically significant difference was observed (p= 0.117). In the group with a CD20+ lymphocyte count > 104.5/ mm^3, the 2-year survival rate was 79% and the 5-year survival rate was 69%. Conversely, in the group with a CD20+ lymphocyte count ≤ 104.5/ mm^3, the 2-year survival rate was 8% and the 5-year survival rate was 35.5%.

The median OS (months) according to post-vaccination CD-20+ lymphocyte levels was found to be statistically significant (p< 0.001). In the group with a CD-20+ lymphocyte count > 94.5/mm^3, the median OS was not reached. Conversely, in the group with a CD-20+ lymphocyte count ≤ 94.5/ mm^3, the median OS was determined to be 24.73 months (95% CI: 17.09-32.37). In the group with a CD-20+ lymphocyte count > 94.5/mm^3, the 2-year survival rate was 100% and the 5-year survival rate was 90%. In contrast, in the group with a CD-20+ lymphocyte count ≤ 94.5/mm^3, the 2-year survival rate was 57.1%, whereas the 5-year survival rate was 14.3%.

The median OS (months) by post-vaccination lymphocyte count was statistically significant (p= 0.021). In the group with a lymphocyte count >1650/mm^3, the median OS could not be achieved. Conversely, in the group with a lymphocyte count ≤ 1650/mm^3, the median OS was calculated to be 10 months (95% CI: 21.43-48.76). In the group with a lymphocyte count >1650/mm^3, the 2-year survival rate was 90.9% and the 5-year survival rate was 79.5%. In contrast, in the group with a lymphocyte count ≤ 1650/mm^3, the 2-year survival

rate was 71.6%, whereas the 5-year survival rate was 31.3%. The median OS (months) according to post-vaccination albumin level was statistically significant (p= 0.001). The group with a post-vaccination albumin level of > 40.5 g/L did not reach the median OS. In contrast, the group with a post-vaccination albumin level ≤ 40.5 g/L had a median OS of 33 months (95%CI: 22.72-31.94). The 2-year survival rate for the group with a post-vaccination albumin > 40.5 g/L was 93.8%, and the 5-year survival rate was 93.8%. In the group with a post-vaccination albumin ≤ 40.5 g/L, the 2-year survival rate was 66%, while the 5-year survival rate was 22%. The median OS (months) by post-vaccination LN size was statistically significant (p< 0.001). In the group with LN size > 8.5 mm, the median OS could not be reached. Conversely, in the group with an LN size ≤ 8.5 mm, the median OS was determined to be 26.9 months (95% CI: 10.06-43.73). In the group with LN size > 8.5 mm, the 2-year survival rate was 100% and the 5-year survival rate was 9%. In contrast, in the group with LN size ≤ 8.5 mm, the 2-year survival rate was 62.5%, whereas the 5-year survival rate was 20.8%.

The median PFS time (months) by post-vaccination LN size was statistically significant (p= 0.021). In the group with an LN size > 8.5 mm, the median PFS was 65.5 months (95% CI: –). In the group with an LN size ≤ 8.5 mm, the median PFS was 23.53 months (95% CI: 8.18-38.88). Additionally, in the group with an LN size > 8.5 mm, the 2-year PFS was 88.9%, and the 5-year PFS was 57.3%. In contrast, in the group with an LN size ≤ 8.5 mm, the 2-year PFS was 44.4%, while the 5-year PFS was 2%.

The median PFS was determined to be 49.76 months (95% CI: 28.02-71.50). Furthermore, the 2-year PFS rate were 73.9%, while the 5-year PFS rate was 41.1%, respectively.

DISCUSSION

To date, there are no new safety concerns regarding COVID-19 vaccines for cancer patients in general or for cancer patients treated with immune checkpoint inhibitors (ICIs). However, uncertainties persist.[10,13,14,15] Therefore, our study aimed to investigate the effects of immune therapy on vaccines, immune responses, and AEs. To this end, we conducted a prospective control group study.

It is important to note that due to the voluntary participation of patients and healthy individuals within a specific time frame, there was a coincidental imbalance in the gender distribution between the two groups. Specifically, 90% of the patients in the study group were male, whereas 47.4% of the healthy volunteers in the control group were male. These differences were statistically significant.

Relevant literature reports suggest that the female sex may exhibit a stronger immune response, leading to better vaccine response and potentially more AEs.[16,17] In our study, the coincidental overrepresentation of male patients in the study group was considered to partially mitigate confounding effects associated with sex.

mRNA vaccine is generally recommended for cancer patients.[18] Sinovac vaccine was approved first in our country, followed by BioNTech® mRNA vaccine approximately one year later.

While the majority of patients received Sinovac vaccine as the first dose, BioNTech vaccine constituted half of the second dose and 84.4% of the third dose. According to the Health Practice Circular, Nivolumab is approved for reimbursement in our country for the treatment of lung cancer, malignant melanoma, renal cell carcinoma and lung cancer. Therefore, these cancer types were included in our study and nivolumab was the most commonly used agent.

While there is concern that cancer patients, who are generally immunosuppressed, may have lower vaccine antibody responses than the healthy population, studies have shown that patients receiving immunotherapy exhibit similar vaccine antibody responses to healthy volunteers.[19] In line with these results, our study did not find any differences in vaccine antibody responses between the patient group and healthy volunteers. Despite the fact that the patient group in our study underwent intensive oncological treatment prior to immunotherapy, receiving an average of 14.19±16.1 (med: 8) cycles of immunotherapy, and the majority of them had advanced cancer at the time of diagnosis, their equivalent vaccine antibody response to healthy

volunteers highlights the unique efficacy of immunotherapy compared to other cancer treatments. Interestingly, in our study, 7 patients receiving immunotherapy had elevated antibody levels prior to COVID-19 vaccination. There are two potential explanations for this observation. First, these patients might have experienced an asymptomatic SARS-CoV-2 infection before their vaccination, which could have resulted in pre-existing antibodies. Second, immunotherapy itself may modulate the immune system in a way that enhances baseline antibody production. This phenomenon has been reported in previous studies, suggesting that immune checkpoint inhibitors can lead to increased immune activity and autoantibody production. Further investigation is needed to better understand these mechanisms and their clinical implications for cancer patients undergoing immunotherapy.

The study has the limitations of having a single center as the data source, small sample size, including a mix of different cancer types, and the absence of randomization in the study design.

One of the study's limitations is the difference in follow-up durations between the patient and control groups, with the control group followed for 6 months and the patient group for 12 months. This difference arose due to logistical challenges during the COVID-19 pandemic, particularly in maintaining long-term follow-up for healthy volunteers. However, we believe this limitation did not significantly impact the reliability of our findings, as key immune responses were observed within the first few months post-vaccination. The primary outcomes, including antibody levels and lymphocyte subset changes, were evaluated consistently during the initial follow-up period in both groups.

Another limitation of this study is the potential for undetected asymptomatic COVID-19 infections in both the patient and control groups. Although prior COVID-19 history was collected, some individuals may have experienced asymptomatic infections that were not reported or diagnosed. This could have influenced immune response measurements, particularly antibody levels. Future studies incorporating more comprehensive testing strategies, such as serology tests, are needed to address this issue.

IrAEs are common, affecting up to 76% of treated patients 3. Among these, thyroid-related irAEs are the most frequently observed endocrine toxicity associated with ICI therapy.[20,21,22,23] Two large observational studies focusing on thyroid irAEs reported rates of such events associated with ICI therapy ranging from 42% to 53%.[24,25] Similarly, in our study, we observed a similar rate of 40.6% of thyroid-related irAEs after vaccination, and thyroid-related irAEs were the most common AEs both alone and in combination with other irAEs.

In our study group, immune-related side effects (iRAe); colitis, hepatitis, carditis, activation of underlying autoimmune disease and thyroiditis were all seen. Two of these, carditis and hepatitis iRAe, were noteworthy findings in terms of their occurrence in our small patient population. Carditis, which carries a serious mortality risk[26] and is a very rare side effect, occurred in one case with lung cancer. Immune-related hepatitis has been reported at varying rates.[27,28] Hepatitis iRAe was also detected in two of our cases.

A study conducted by Waissengrin et al. reported that the incidence of irAEs in cancer patients remained consistent with the rates observed before COVID-19 vaccination.[10] Similarly, Chen et al. conducted a study that found no evidence of an increased risk of new or worsened irAEs following COVID-19 vaccine administration in cancer patients receiving immune checkpoint inhibitors.[29]

In our study, most patients experiencing AEs, with only a few exceptions, showed varying degrees of increase in lymphocyte counts. Among patients with thyroiditis and underlying autoimmune disease activation, the greatest increase was observed in CD4+ lymphocyte counts, while decreases were observed in other lymphocyte lineages. A study by Yasuda et al. investigating irAEs after PD-1 treatment in a mouse model suggested that activated T cells are responsible for destructive thyroiditis and that CD4+ T lymphocytes are the most prevalent in thyroiditis AEs, followed by CD8+ T lymphocytes.[30] It is also known that hepatotoxicity due to ICI treatment is caused by CD8+ lymphocytes, which directly and indirectly damage the liver through cytokines.[28] In our study, while other lymphocyte subgroups decreased in

the thyroiditis+hepatitis case, only an increase was observed in CD8+ lymphocytes (144/mm^3 [%26]). Although lymphocyte subset analyses were performed before the first vaccination and after at least one dose of the COVID-19 vaccine, we did not analyze lymphocyte subsets specifically before and after the development of immune-related adverse events (irAEs) due to limited availability of laboratory kits. The lack of adequate kits prevented more detailed evaluations at different time points during the follow-up period.

For the management of irAEs, steroid treatments were administered when necessary, especially in patients with thyroid dysfunction, colitis, and dermatitis. Further studies with more comprehensive resources are warranted to better understand the immune modulation associated with irAEs.

It is known that mRNA COVID-19 vaccines can cause lymphadenopathy in draining lymph nodes.[31] It has been recommended that the vaccine be administered contralateral to the affected breast to avoid confusion in the follow-up of breast cancer patients.[32] A study demonstrating the durability of the B cell response after mRNA vaccination showed that CD19+ and CD20+ lymphocytes were found in both lymph node germinal centers and blood samples, along with other B cell markers, as detected by flow cytometry.[33] In our study, the increase in CD19+ and CD20+ lymphocytes was associated with a greater number of affected lymph node regions. In addition, a positive correlation was found between the increase in the size of the affected lymph nodes (mm) and the increase in the number of CD20+ lymphocytes after the first two vaccine doses.

Reactive lymph nodes were identified during follow-up imaging scans and were predominantly observed in the axillary and cervical regions. However, no significant relationship was observed between the location of the reactive lymph nodes and the site of vaccine administration. These findings suggest that the lymph node enlargement is more likely due to a generalized immune response rather than a localized reaction to the vaccine. It has been found that LDH levels are inversely proportional to the response to checkpoint inhibitors and that high LDH levels are associated with high tumor burden.[34]

In our study, we observed a positive correlation between the presence of reactive lymph node involvement, the number of lymph node regions involved, and LDH levels after the first two vaccine doses. However, it is worth noting that there are studies showing that the presence of reactive lymph nodes in patients receiving immunotherapy may be a positive indicator of treatment response.[35]

Although we did not observe a significant correlation between peripheral blood lymphocyte levels and immune response in our study, peritumoral infiltrative lymphocytes (TILs) within the tumor microenvironment may independently influence the effectiveness of immunotherapy. It is well established that TIL presence is associated with better treatment outcomes. However, due to the lack of tumor sample collection in our study, we were unable to directly evaluate this correlation.

This limitation may explain why stable blood lymphocyte levels were observed post-vaccination despite high immunotherapy response rates. TIL activity may play a more substantial role in immune modulation than peripheral blood lymphocyte changes.

The observed changes in lymphocyte subsets in patients who developed immune-related adverse events (irAEs) after vaccination may be attributed to multiple factors. First, it is possible that the immune-modulating effect of the COVID-19 vaccine amplified pre-existing immune responses in these patients. Vaccines, especially mRNA-based ones, are known to activate both innate and adaptive immunity, which could lead to fluctuations in lymphocyte subsets.

Second, the baseline immune status of patients undergoing immunotherapy may have influenced these changes. Immune checkpoint inhibitors (ICIs) can modulate the activity of various lymphocyte subsets, and this interaction with vaccination may have contributed to the variations observed. Further studies are needed to clarify the interplay between immunotherapy and vaccination on lymphocyte dynamics.

The correlation of the increase in LDH, which is a marker of poor prognosis, with reactive lymph nodes was an unexpected result of our study. In addition, the increase in LDH levels after vaccination

compared to healthy volunteers suggests that LDH and reactive lymph nodes should be evaluated as a different situation in the combination of vaccination and immunotherapy. Our study ultimately demonstrated that both the increase in lymph node size and LDH levels have an effect on survival.

The immune therapy responses in the patient group were evaluated at the end of the third, sixth, and first years after vaccination. The results showed a gradual increase in the response rate after vaccination (12.5%, 17.9%, and 33.3%, respectively). In contrast, the rate of progressive disease was higher at 6 months (35.7%) than at 3 months (25%), but the lowest rate was observed at 1-year follow-up (16.7%). A large study conducted in China with 2048 patients compared vaccinated and unvaccinated individuals. This study found that the inactivated SARSCoV-2 virus vaccine (BBIBP-CorV vaccine) did not negatively impact the effects of immunotherapy or increase AEs. Therefore, there is no need to interrupt immunotherapy following vaccination.

Compared with the unvaccinated subgroup, vaccinated patients had a higher rate of stable disease (45.7% vs. 38.1%; p= 0.003) and a higher disease control rate (DCR, 72.2% vs. 67.0%; p= 0.026). However, the partial response rate was slightly lower in t he vaccinated patients (20.2% vs. 24.7%; p= 0.031).[36]

Our study investigated whether there is a relationship between vaccine type and immune therapy response. Our results revealed no significant relationship between different vaccine regimens (with and without BioNTech) and 6-month and 1-year immunotherapy responses. However, at 3 months, patients who received a regimen that included the BioNTech vaccine had a higher rate of partial response to immune therapy. Interestingly, all patients who achieved complete response (CR) and partial response (PR) at 3 months received a regimen including the BioNTech vaccine, while the rate of progressive disease was three times higher in patients who received a regimen without the BioNTech vaccine. In comparison to the study conducted by Mei et al.[36], the higher gradual increase in partial response rate in our study after vaccination might be attributed to the use of both inactivated and mRNA vaccines with higher immunogenicity, whereas Mei et al.'s study only utilized the inactivated vaccine. The gradual increase in partial response rates over time aligns with the typical observations of immune therapies, where treatment efficacy becomes more evident as time progresses. We hypothesized that the observed gradual increase in partial response rates, along with the higher rate of progressive disease at 6 months and its subsequent decrease at 1 year in our study, could be attributed to the delayed effects of immune therapy responses and the higher proportion of mRNA vaccine doses in the second and third vaccinations.

It is believed that irAEs may indicate a positive response to treatment.[37] However, studies on this topic have yielded conflicting results. For example, Horvat et al conducted a study and reported that the occurrence of irAEs, regardless of their severity, did not affect OS or time to treatment failure.[38]

On the other hand, Freeman-Keller et al conducted a study specifically on patients with melanoma and found that OS was higher in patients who experienced irAEs, regardless of their severity, than in those who did not experience them.[39] In our study, we evaluated patients who developed immune AEs after vaccination. Although there was no significant difference in treatment response at the 3-month and 6-month intervals after vaccination based on the occurrence of AEs, we observed that patients who experienced AEs had a better immune therapy response at 1 year.

In a study by Julia et al., an increase in peripheral blood CD8+ T lymphocytes was evaluated as a biomarker, and a decrease in CD4 and CD8+ T cell levels was associated with disease progression.[40]

Previous studies have shown that patients who respond to immunotherapy have lower baseline circulating T cell levels compared with nonresponders.[41,42,43] In our study, we observed a negative correlation between the 3-month post-vaccination diameter of target lesions and the CD8+ and CD20+ lymphocyte counts. In addition, there was a negative correlation between the 6-month post-vaccination diameter of target lesions and the CD20+ lymphocyte counts. Furthermore, we found a negative correlation between the 3-month post-vaccination SUVmax of the target lesion and

the CD8+ lymphocyte count. These results are consistent with the existing literature on tumor and circulating lymphocyte types in patients receiving immunotherapy. In our study, the BioNTech vaccine was predominantly administered to both the healthy volunteer and patient groups across all doses. However, the patient group had a higher proportion of individuals receiving the Sinovac inactivated vaccine for the first and second doses than the healthy volunteers. Nevertheless, no significant difference was observed in vaccine antibody levels between the healthy and patient groups. In a study by Oosting et al., which compared the efficacy of mRNA vaccines and immunotherapy, no difference in vaccine response was found between the healthy volunteer group and the patient group receiving immunotherapy for solid organ cancer.[19]

Additionally, a meta-analysis including data from 10.865 patients, 2.477 of whom received immunotherapy, reported no difference in vaccine response between patients receiving ICI treatment and healthy volunteers or patients with cancer who did not receive ICI.[44]

Among the significant factors for predicting mortality, "pre-vaccination and post-vaccination CD20 levels", "post-vaccination CRP", "post-vaccination LDH", "post-vaccination LN size", "post-vaccination peripheral blood total lymphocyte count", and "post-vaccination albumin level", which we found to be relevant for contributing to the existing literature, were compared with available data in the literature.

In studies of patients with ovarian colorectal cancer, CD20+ lymphocytes, similar to CD8+ T lymphocytes, have shown favorable prognostic effects.[45,46] In our study, we hypothesized that CD8+ and CD20+ lymphocytes in peripheral blood may indirectly reflect the content of tumor-infiltrating lymphocytes and may influence the prediction of treatment response and survival.

In this respect, we showed that the measured CD20+ lymphocyte count was a significant predictor of median OS. In the group with a post-vaccination CD20+ lymphocyte count of >94.5/mm^3, the 2-year survival rate was 100% and the 5-year survival rate was 90%. In contrast, in the group with a post- vaccination CD20+ lymphocyte count of ≤ 94.5/mm^3, the 5-year survival rate was 14.3%.

In the literature, the relationship between CRP levels and mortality in cancer patients has been shown, as well as the relationship between immune response.[47,48] Similarly, in our study, it was shown that CRP levels of 11.5 mg/L and above were significant predictors of mortality, while a negative correlation was shown between CRP levels and CD4+ and CD20+ lymphocyte levels. High LDH levels are associated with poor outcomes in cancer patients. A relationship between LDH levels and survival has been observed in melanoma and various other tumor types. Additionally, patients with high LDH levels tend to derive less benefit from checkpoint inhibitors than those with normal LDH levels.[34] In our study, post-vaccination LDH values of ≥ 211 U/L were found to be significant predictors of mortality.

Reactive lymph node enlargement may be evaluated as "pseudo-positive" enlarged nodes and is considered an indicator of an activated immune system. Spitzer and colleagues discovered that when migration from primary lymphoid organs to the tumor environment is suppressed, the response to immunotherapy is poor.[49] In our study, a positive association was found between lymph node size (> 8.5 mm) and 2- and 5-year survival rates (OS 100% and 92.9%; PFS 88.9% and 57.3%, respectively).

Our study showed that reactive lymph node size of 8.5 mm and above after vaccination is an important factor associated with reduced mortality. These results provide important data for the current literature. In a study conducted by Pan et al., it was observed that a low peripheral lymphocyte count and high LDH levels had a negative impact on treatment response and survival in head and neck cancer patients treated with immune checkpoint inhibitors.[50] Consistent with these results, our study identified a low peripheral lymphocyte count, particularly values ≤ 1650/mm^3, as a significant predictor of mortality. Furthermore, patients with a post-vaccination lymphocyte count of >1650/mm^3 exhibited a 2-year survival rate of 90.9% and a 5-year survival rate of 79.5%, whereas those with a lymphocyte count of ≤ 1650/ mm^3 had a 2-year survival rate of 71.6% and a 5-year survival rate of 3%.

A meta-analysis involving 36 studies and 8406 cancer patients reported that lower albumin levels,with

a cut-off value of 3.5 g/dL, were associated with an increased risk of death.[51] Similarly, in our study, a post-vaccination albumin level of ≤ 40.5 g/L was found to be significant in predicting mortality. Patients with an albumin level > 40.5 g/L had a 2-year survival rate of 93.8%, and a 5-year survival rate of 93.8%, whereas those with an albumin level ≤ 40.5 g/L had a 2-year survival rate of 66% and a 5-year survival rate of 22%, demonstrating a significant difference.

Conclusion

This study provides real-world evidence on the interaction between COVID-19 vaccination and immunotherapy in cancer patients. Including both inactivated and mRNA vaccines, alongside healthy volunteers as controls, enhances its clinical relevance.

Our findings show that COVID-19 vaccines elicit a comparable immune response in cancer patients and healthy individuals, with no increase in immune-related adverse events (irAEs). The study highlights a link between reactive lymph nodes, immune responses, and survival outcomes. Patients who developed irAEs demonstrated better long-term treatment responses.

The type of vaccine also influenced outcomes, with the BioNTech arm showing superior responses at three months post-vaccination. Peripheral CD8+ and CD20+ lymphocytes correlated with tumor characteristics and survival, emphasizing the overlooked role of B lymphocytes.

In conclusion, this study supports the safe integration of COVID-19 vaccination with immunotherapy and underscores the need for further research on localized immune responses in predicting treatment outcomes.

This study has several important contributions to the existing literature. First, it provides real-life data through a prospective and non-interventional design. No similar study has been found in the literature that examines the bilateral effects of COVID-19 vaccines and immunotherapy.

Furthermore, the inclusion of healthy volunteers as the control group and the long-term follow-up of both groups made this study valuable. Another distinguishing feature is that the study included both inactivated and mRNA COVID-19 vaccines, which sets it apart from the previous literature on immunotherapy and vaccine effects.

This study clearly demonstrates the relationship between peripheral blood T and B lymphocytes, immune responses, and AEs. Additionally, it highlights the relationship between reactive lymph nodes and immune response.

Although the majority of patients in the patient group received inactivated vaccines, the absence of a difference in antibody levels between the patient and healthy volunteer groups indicated the influence of immunotherapy on vaccine efficacy. Moreover, the study revealed that the type of vaccine had an impact on the immune treatment response, with the BioNTech arm showing better results at post-vaccination 3 months compared to the arm without BioNTech.

In terms of tumor characteristics, this study highlights the relationship between the diameters and SUVmax values of the target lesions and CD8+ T lymphocytes. However, CD20+ B lymphocyte levels were more significant in terms of mortality status and median survival. This result emphasizes the significance of peripheral B lymphocytes, which have often been overlooked in studies focusing on T lymphocytes.

Notably, the study did not show an increase in AEs associated with co-administration of immunotherapy and COVID-19 vaccination. In fact, a decrease in the counts of peripheral T and B lymphocyte subsets was observed in patients without AEs compared to those with AEs. Demonstrating the variation in peripheral lymphocyte subsets by AE type is also a notable result. Additionally, this study demonstrated that patients who developed irAEs exhibited better treatment responses at the end of the first year after vaccination.

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Correspondence:

Dr. Arif Hakan ÖNDER

Saglik Bilimleri Universitesi

Antalya Egitim ve Arastirma Hastanesi

Tibbi Onkoloji Bolumu

Varlik Mahallesi, Kazim Karabekir Sokak

Muratpasa, ANTALYA / TURKIYE

Tel: (+90-539) 482 21 24

e-mail: dr_hakanonder@hotmail.com

ORCIDs:

Arif Hakan Onder	0000 0002 0121 5228
Kübra Demir Onder	0000 0002 4164 5118
Yesim Cekin	0000 0003 4393 5618
Banu Oztürk	0000 0003 0290 8787
Erdal Kurtoglu	0000 0002 6867 6053