REGULAR ARTICLE

blood advances

Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with B-cell non-Hodgkin lymphoma

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Key Points

- Patients with B-NHL treated with an anti-CD20 antibody are unlikely to achieve humoral response to BNT162b2 mRNA COVID-19 vaccine.
- Longer time since last exposure to anti-CD20 antibodies predicts a higher response rate and elevated antibody titer.

Patients diagnosed with B-cell non-Hodgkin lymphoma (B-NHL), particularly if recently treated with anti-CD20 antibodies, are at risk of severe COVID-19 disease. Because studies evaluating humoral response to COVID-19 vaccine in these patients are lacking, recommendations regarding vaccination strategy remain unclear. The humoral immune response to BNT162b2 messenger RNA (mRNA) COVID-19 vaccine was evaluated in patients with B-NHL who received 2 vaccine doses 21 days apart and compared with the response in healthy controls. Antibody titer, measured by the Elecsys Anti-SARS-CoV-2S assay, was evaluated 2 to 3 weeks after the second vaccine dose. Patients with B-NHL (n = 149), aggressive B-NHL (a-B-NHL; 47%), or indolent B-NHL (i-B-NHL; 53%) were evaluated. Twenty-eight (19%) were treatment naïve, 37% were actively treated with a rituximab/obinutuzumab (R/Obi)–based induction regimen or R/Obi maintenance, and 44% had last been treated with R/Obi >6 months before vaccination. A seropositive response was achieved in 89%, 7.3%, and 66.7%, respectively, with response rates of 49% in patients with B-NHL vs 98.5% in 65 healthy controls ($P < .001$). Multivariate analysis revealed that longer time since exposure to R/Obi and absolute lymphocyte count $\geq 0.9 \times 10^3/\mu L$ predicted a positive serological response. Median time to achieve positive serology among anti-CD20 antibody-treated patients was longer in i-B-NHL vs a-B-NHL. The humoral response to BNT162b2 mRNA COVID-19 vaccine is impaired in patients with B-NHL who are undergoing R/Obi treatment. Longer time since exposure to R/Obi is associated with improved response rates to the COVID-19 vaccine. This study is registered at www.clinicaltrials.gov as #NCT04746092.

Introduction

Despite firm governmental epidemiological restrictions aimed at controlling the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, COVID-19 continues to spread. The emergence of new mutations characterized by increased infectivity rate and, potentially, a higher mortality rate[1-4] emphasizes the need for early introduction of a rapid vaccination program. However, data regarding the effectiveness of anti-SARS-CoV-2 vaccines in the presence of B-cell non-Hodgkin lymphoma (B-NHL), especially in patients recently treated with a B-cell–depleting therapy, are lacking, and recommendations regarding their use in this setting are still insufficient. Moreover, recent data suggest that hemato-oncological patients, including those with B-NHL, are at increased risk of developing severe COVID-19[5,6] and may serve as "sustained viral reservoirs," promoting the development of new, potentially more

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aggressive mutations.[7,8] Thus, preventing COVID-19 infection, or at least attenuating disease severity in these patients, is of utmost importance.

Recently, the US Food and Drug Administration has granted approval to several anti-SARS-CoV-2 vaccines, including the BNT162b2 and mRNA-1273 messenger RNA (mRNA) vaccines, which are now recommended to prevent COVID-19. BNT162b2 and mRNA-1273 are lipid nanoparticle–encapsulated mRNA-based vaccines, that encode the full-length S protein of SARS-CoV-2.[9,10] These vaccines have shown high efficacy in preventing symptomatic SARS-CoV-2 infection, but hemato-oncology patients were excluded from the clinical trials of COVID-19 vaccine.[11,12] In the current study, we investigated the humoral response to SARS-CoV-2 vaccine in patients with B-NHL and looked at factors affecting the response rate to the vaccine, with the intention of providing data that can be used to establish evidence-based recommendations regarding vaccination strategy in this unique population of patients.

Methods

In the current prospective study, we investigated the efficacy of BNT162b2 mRNA COVID-19 vaccine by Pfizer (hereinafter, COVID-19 vaccine) in patients with B-NHL, diagnosed and followed up at the Tel Aviv Sourasky Medical Center, who were vaccinated against SARS-CoV-2 as part of the Israeli national vaccination program. The primary end point of the study was the proportion of subjects acquiring anti-SARS-CoV-2S antibodies (Abs). The study was approved by Tel Aviv Sourasky Medical Center's Institutional Review Board. All patients provided informed consent.

Patient population

The study included patients aged ≥18 years diagnosed with B-NHL, including diffuse large B-cell lymphoma (DLBCL) and primary mediastinal B-cell lymphoma (forming a subgroup referred to as "aggressive [a]-NHL") and follicular lymphoma and marginal zone lymphoma (forming the "indolent [i]-NHL" subgroup). All patients were observed or treated at the Hematology Division of the Tel Aviv Sourasky Medical Center during the study period (20 December 2020 to 10 March 2021). All patients included in the study had 2 consecutive doses of the BNT162b2 mRNA COVID-19 vaccine, administered 21 days apart. The patients were classified into 3 groups: (1) treatment-naïve patients (patients with indolent lymphoma under "watch-and-wait" management); (2) actively treated patients who were receiving treatment with anti-CD-20 Ab (rituximab/obinutuzumab [R/Obi])–based chemoimmunotherapy (induction or salvage), R monotherapy, or R/Obi maintenance at the time of vaccination (because exposure to rituximab induces severe B-cell depletion for at least 6 months,[13] patients who completed treatment up to 6 months before vaccination were included in this group); and (3) patients who had completed chemoimmunotherapy/immune monotherapy/maintenance >6 months before vaccination. Age-compatible, healthy volunteers, aged ≥18 years, who had received 2 consecutive COVID-19 vaccine doses, served as controls.

Study design

According to our department's policy, patients receiving induction or salvage antilymphoma treatment every 21 to 28 days were advised to be vaccinated 7 to 14 days after their most recent course of chemoimmunotherapy/immunotherapy, whereas patients receiving R/Obi maintenance every 8 to 12 weeks were advised to receive their first and second vaccinations 30 and 51 days after the latter maintenance cycle, respectively. These time points were chosen because we sought to obtain the longest possible interval between R/Obi doses. Treatment-naïve patients and those who completed therapy at least 6 months before vaccination were given no specific recommendations regarding the timing of vaccination.

Dates of both vaccination doses were recorded. All participants (patients and healthy controls) underwent serology tests, measuring their humoral response to COVID-19 vaccine 14 to 21 days after the second vaccine dose. Demographic and clinical data, focusing on histological diagnosis, treatment regimen, treatment status and timing, disease status, response to treatment, and absolute lymphocyte count (ALC) at the time of vaccination were collected from the patients' electronic medical records. Adverse events (AEs) reported within the first 7 days after each of the 2 vaccine doses were recorded.

Assessment of serological response

Serum samples were analyzed by using the Elecsys Anti-SARS-CoV-2S assay,[14] performed on the Cobas e601 (Roche Diagnostics) enzyme-linked immunosorbent assay reader for quantitative detection of Abs, predominantly IgG, aimed at the SARS-CoV-2 S protein receptor binding domain. This assay has a measurement range of 0.40 to 250 U/mL, with a concentration of <0.80 U/mL considered to be a negative result and ≥0.80 U/mL considered to be positive. When results exceed the upper limit of the measurement range (reported as >250 U/mL), samples are diluted by 1:10 or 1:100, depending on the dilution range that is required. To ensure that none of the patients had been exposed to SARS-CoV-2 recently, we performed an additional test for the presence of Abs to the SARS-CoV-2 nucleocapsid protein, using the Elecsys Anti-SARS-CoV-2 assay,[15] performed on the Cobas e601 (Roche Diagnostics).

Statistics

Continuous variables were described as the median and range/interquartile range of observations. Categorical data were described with contingency tables including frequency and percentages. Conversion of continuous variables into categorical variables was based on both frequency distributions and clinical familiarity with impact factors on the response variable. The χ^2 test for association was performed to check marginal relationships. Receiver operating characteristics analysis was performed to define the optimal cutoff point for achieving positive serology from the last anti-CD20 treatment. Pearson's χ^2 test, Fisher's exact test, and univariate Cox regression were used to study the crude association between the studied predictors and the vaccine response rate. The Mann-Whitney U test was used to compare medians of Ab concentration levels (titers). The multivariate Cox regression analysis was performed, using the backward method ($P < .1$, was used as the criterion for removal), to identify independent predictors for the response rate. Two-sided $P < .05$ indicated statistically significant differences. Variables with trending or significant association to response rate or those known to be of important clinical significance were tested in the multivariate model. The Kaplan-Meier method and Cox regression analysis were used to calculate the hazards ratio (HR) at different time points from the last treatment. SPSS software (IBM SPSS Statistics for Windows, version 27, 2017; IBM Corp., Armonk, NY) was used for all statistical analyses.

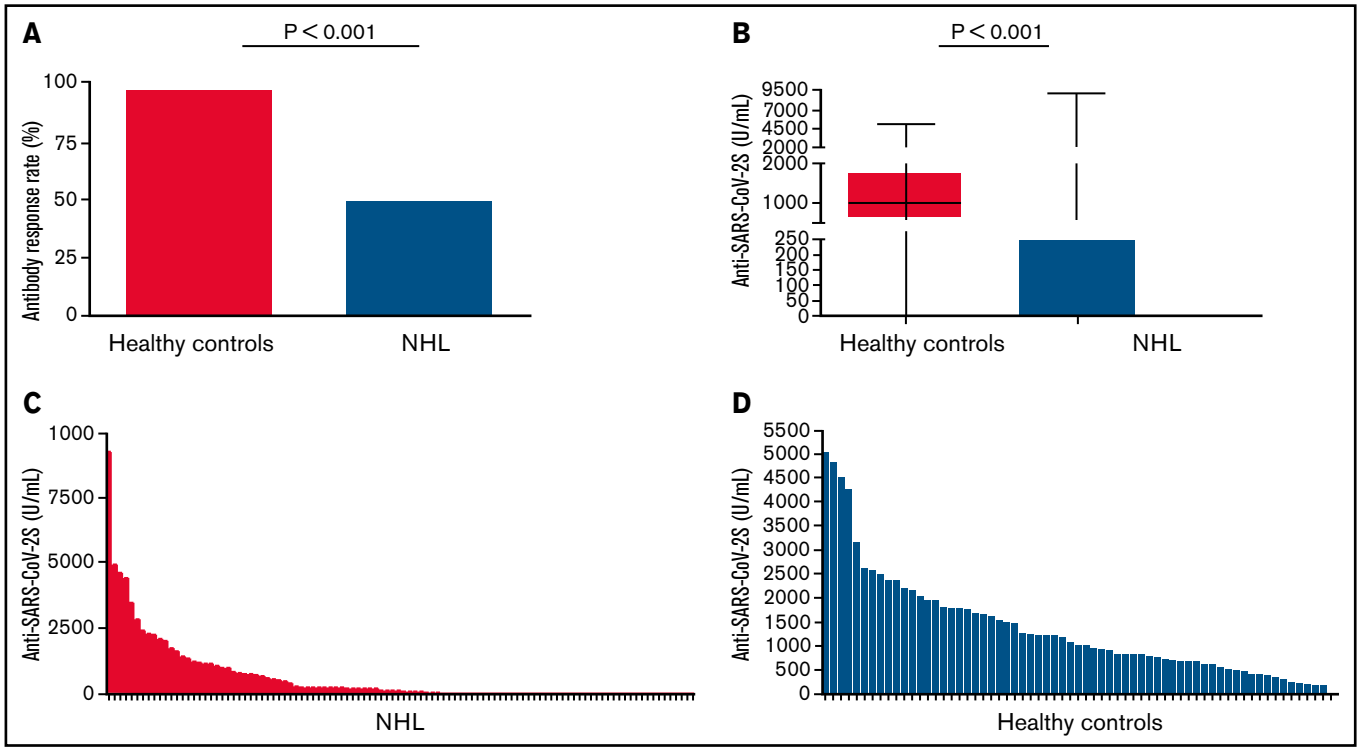

Figure 1. Serological response rates to COVID-19 vaccine. (A) Percentage of response rates in patients with B-NHL vs age-compatible, healthy controls. (B) Median values and range of anti-SARS-CoV-2 Ab titer levels in patients with B-NHL vs healthy controls. Mean titers ± standard deviation for each group are presented. (C-D) Distribution of anti-SARS-CoV-2 Ab titer levels among B-NHL patients and healthy controls. In panel C, each bar represents 1 patient with B-NHL, in panel D, each bar represents 1 healthy control.

Table 1. Patient demographics and disease characteristics

Variables	n (%)/median (range)
Age (median, range), y	64 (20-92)
Age ≤60 y	58 (38.9)
Sex, male	88 (59.1)
Time from diagnosis to vaccination, mo	22 (0.2-354)
Diagnosis	
a-B-NHL	69 (47)
i-B -NHL	80 (53)
ALC ($\times 10^3/\mu L$) mean ± standard deviation, median (range)	1.7 ± 2.9, 1.08 (0.26-30.5)
Treatment status	
Treatment naïve (watch and wait)	28 (18.8)
Actively treated (≤6 mo from last anti-CD20 therapy)	55 (37)
Completed treatment >6 mo	66 (44.2)
Any exposure to anti-CD20 Abs	121 (81.2)
Median time (mo) from last anti-CD20 Ab therapy (range)	7.3 (0-204)
Disease status (for 111 evaluable patients of 121 treated patients)	
CR	98 (88.3)
PR	6 (5.4)
SD	3 (2.7)
PD	4 (3.6)

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Results

Patient characteristics

One hundred and forty-nine patients with B-NHL (88 [59%] men, median age 64 [range 20-92] years), and 65 healthy controls (29 [45%] men, median age 66 [range 25-83] years) were included in the study. Patients' characteristics are presented in Table 1. Eighty (53%) patients were diagnosed with i-B-NHL and 69 (47%) with a-B-NHL. Twenty-eight (19%) patients were treatment naïve, 39 (26%) were treated with R/Obi-containing chemoimmunotherapy or R monotherapy, 16 (11%) were currently receiving anti-CD20 Ab maintenance, and 66 (44%) had completed therapy >6 months before vaccination. Among all patients who were treated with anti-CD20 Abs, the median time from last R/Obi cycle to vaccination was 7.3 (range, 0.1-204) months: 2.3 (range, 0-5.6) months for patients in active treatment, 0.5 (range, 0-3.8) months for patients receiving R/Obi maintenance, and 18 (range, 6-204) months for patients who had completed treatment >6 months before vaccination. Mean ALC ± standard deviation at the time of vaccination was 1.7 × $10^3/\mu L$ ± 2.9 × $10^3/\mu L$, with a median count of 1.08 × $10^3/\mu L$ (range, 0.26 × $10^3/\mu L$ to 30.5 × $10^3/\mu L$).

Serologic response

The Ab response to the COVID-19 vaccine was achieved in 73 of 149 (49%) patients with B-NHL included in our cohort, compared with 64 of 65 (98.5%) age-compatible, healthy controls ($P < .001$). Response rates in patients receiving an active anti-CD20 Ab–containing treatment regimen (chemoimmunotherapy or immune

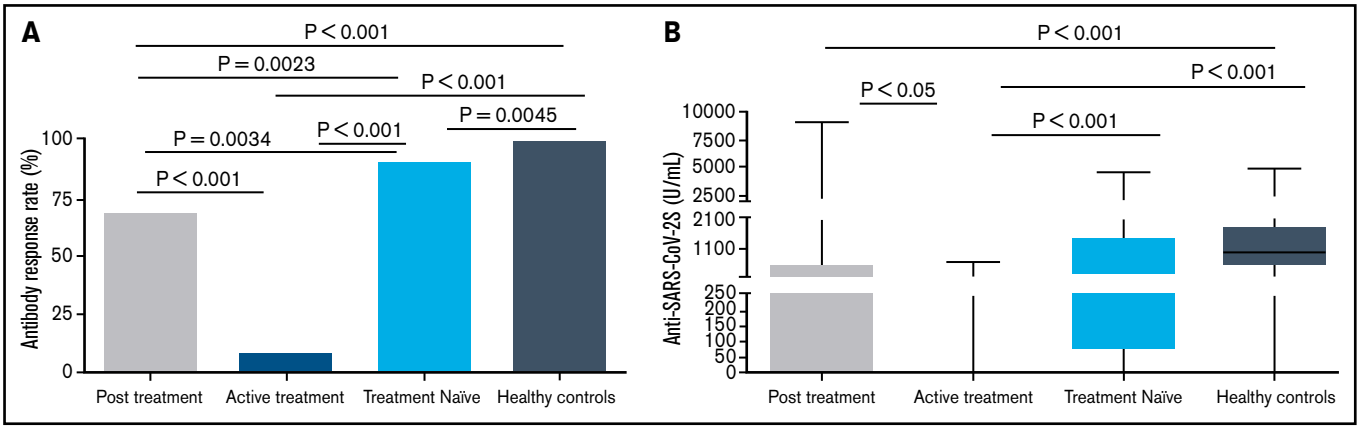

Figure 2. Serological response rates in subgroups of patients with B-NHL. (A) The percentage of seropositive patients in each of the B-NHL patient subgroups: post treatment (patients who completed anti-CD20 Ab containing therapy >6 months before vaccination), active treatment (patients who are under current anti-CD20 Ab therapy or that completed treatment up to 6 months before vaccination), and treatment naïve (patients with i-B-NHL under watch-and-wait management), compared with healthy controls. (B) Shown are titer levels in each of the B-NHL patient subgroups and in healthy controls.

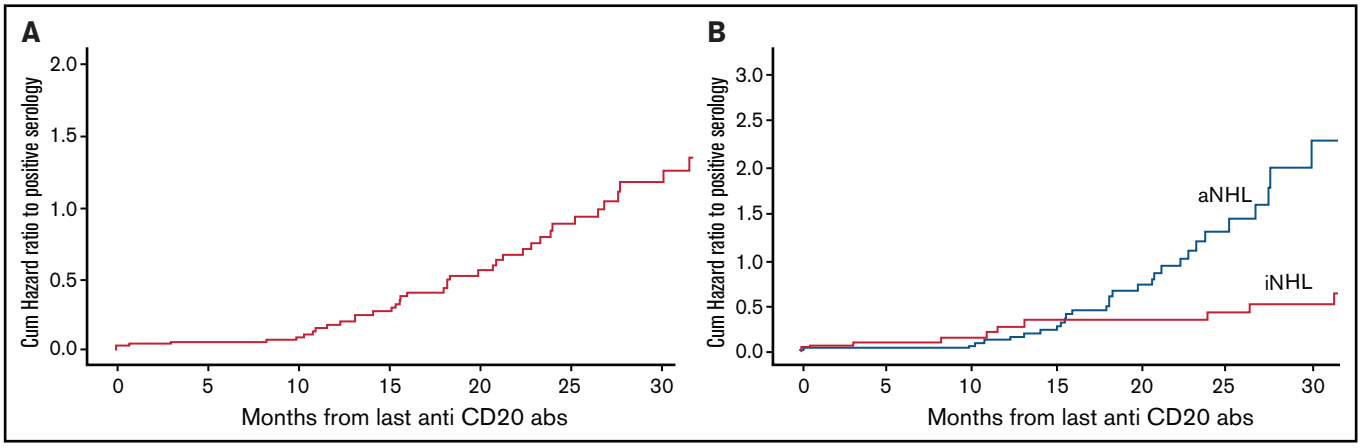

Figure 3. A longer time since last exposure to anti-CD20 Abs is associated with increased seropositivity rates. (A) Hazard ratio (HR) to achieve positive anti-SARS-CoV-2 serology in all patients with B-NHL who were exposed to anti-CD20 Abs is plotted against months from last exposure to anti-CD20 Abs, using a Kaplan-Meier HR curve. (B) HR to achieve positive anti-SARS-CoV-2 serology in patients with a-B-NHL and i-B-NHL who were exposed to anti-CD20 Abs is plotted against months since last exposure to anti-CD20 Abs, using a Kaplan-Meier HR curve.

monotherapy) and in patients currently treated with R/Obi maintenance were 10.3% and 0%, respectively ($P = .24$), both significantly lower than in healthy controls ($P < .001$). Three of 25 (12%) patients who were receiving active anti-CD20 Ab–containing therapy (2 of 3 were at the beginning of treatment at the time of vaccination and completed 2 vaccine doses before the second treatment cycle) and 1 of 30 (3.3%) patients who had completed treatment within 6 months before vaccination attained positive serology ($P = .32$). In treatment-naïve patients with i-B-NHL who received watch-and-wait management and in patients who completed any anti-CD20 Ab–containing therapy at least 6 months before vaccination, response rates were high (89.3% and 66.7%, respectively), compared with patients receiving active or maintenance R/Obi treatment ($P < .0001$). Yet, these rates were still significantly lower than those achieved in age-compatible healthy controls ($P = .007$ for treatment-naïve vs healthy controls and $P < .001$ for patients >6 months from last R/Obi-containing treatment vs healthy controls). Figure 1A shows the response rates in patients with B-NHL vs healthy controls, and Figure 2A shows the response rates in the B-NHL subgroups.

At 6 months from last exposure to anti-CD20 Abs, seropositivity was attained in very few patients. The percentage of seropositive patients began to increase significantly at 9 months from last exposure to R/Obi (based on ROC analysis), reaching 10% at that time point. By 12 months from last exposure to anti-CD20 Abs, 25% of the patients achieved positive serology, and only at 22.3 months from the last R/Obi treatment did 50% of the patients have a positive serological test (Figure 3A). A logistic regression analysis showed that each additional month from last anti-CD20 therapy added a supplementary contribution to the odds of achieving positive serology (HR, 1.1; 95% confidence interval [CI], 1.06-1.13; $P < .001$).

The median time from last exposure to anti-CD20 Abs to the attainment of positive serology was 36 months in patients with i-B-NHL and 19.8 months in patients with a-B-NHL ($P = .031$). Response

Table 2. Univariate analysis evaluating factors predicting positive anti- SARS-CoV-2 serology

Variables		Ab expression, n (%)		Total	*P*	Odds ratio	95% CI
		Positive	Negative				
Analysis of entire cohort (n = 149)							
Age at time of vaccination, y	≤60	29 (50.0)	29 (50.0)	58	.844	1	0.96-1.02
	>60	47 (51.6)	44 (48.9)	91	–	–	–
Sex	Female	32 (52.5)	29 (47.5)	61	.481	1.26	0.66-2.4
	Male	41 (46.6)	47 (53.4)	88			
Disease type	Indolent lymphoma	38 (48.1)	42 (52.5)	80	.87	1.04	0.55-2.00
	Aggressive lymphoma	34 (49.3)	35 (50.7)	69	–	–	–
ALC (111 evaluated patients)	$\leq 0.9 \times 10^3$ /μL	9 (20.5)	35(79.5)	44	**.002**	3.32	1.53-7.21
	$>0.9 \times 10^3$/μL	42(54.5)	35(45.5)	77	–	–	–
Treatment status (detailed)	Treatment naïve	25 (89.3)	3 (10.7)	28	**<.001**	1	
	Active therapy*	4(7.3)	51 (92.7)	55	–	0.039	0.01-0.12
	After therapy	44 (66.7)	22 (33.3)	66	–	4.17	1.13-15.32
Exposure to any anti-CD20 Abs	No	25 (89.3)	3 (10.7)	28	**<.001**	0.08	0.02-0.27
	Yes	48(39.7)	73(60.3)	121	–	–	–
Analysis of patients treated with anti-CD20 abs (n = 121)							
Age in time of vaccination, y	≤60	20 (40.8)	29 (59.2)	49	.832	1.08	0.52-2.27
	>60	28 (38.9)	44 (61.1)	72	–	–	–
Sex	Female	18 (40.0)	27 (60.0)	45	.954	0.98	0.46-2.08
	Male	30 (39.5)	46 (60.5)	76	–	–	–
Disease type	Indolent lymphoma	17 (30.9)	38 (69.1)	55	**.07**	1.98	0.94-4.2
	Aggressive lymphoma	31 (47.0)	35 (53.0)	66	–	–	–
ALC (107 evaluated patients)	$\leq 0.9 \times 10^3$/μL	11(22.0)	39 (78.0)	50	**.006**	3.19	1.37-7.45
	$>0.9 \times 10^3$/μL	27 (47.4)	30(52.6)	57	–	–	–
Time since last anti-CD20 abs (mo)	>6 mo	44(66.7)	22 (33.3)	66	**<.001**	25.5	8.16-77.6
	≤6 mo	4(7.3)	51(92.7)	55	–	–	–
	>9 mo	43 (84.3)	8(15.7)	51	**<.0001**	4.25	3.1-5.43
	≤9 mo	5(7.1)	65(92.9)	70	–	–	–

Bold *P* values indicate statistically significant differences.
*Active therapy refers to all patients treated with anti-CD20 Abs within 6 mo before vaccination, including patients receiving anti-CD20 Ab maintenance.

Table 3. Multivariate analysis evaluating factors predicting anti- SARS-CoV-2 positive serology

Variables	*P*	Odds ratio	95% CI
Analysis of entire cohort (n = 149)			
Age in time of vaccination, y	.01	2.95	1.29-6.73
Prior exposure to anti CD20 abs	.005	0.05	0.06-0.39
Analysis of patients treated with anti-CD20 abs (n = 121)			
ALC $>0.9 \times 10^3$/μL	.036	2.82	1.07-7.4
Time since last anti-CD20 abs (mo)	<.001	1.092	1.04-1.14
Histology: a-B-NHL vs i-B-NHL	.082	2.439	0.89-6.67

Factors included in the analysis of the entire cohort were age, ALC, disease type (a-B-NHL vs i-B-NHL) and prior exposure to anti-CD20 Abs. Factors included in analyses of patients treated with anti-CD20 Abs were age, ALC, disease type (a-B-NHL vs i-B-NHL), and time since last treatment with anti-CD20 Abs.

rates over time remained lower in patients with i-B-NHL, compared with those in patients with a-B-NHL ($P = .034$; Figure 3B).

Of note, none of the patients who developed Abs to the SARS-CoV-2 S protein had anti-SARS-CoV-2 nucleocapsid Abs, thus ruling out the possibility that positive serology was induced by infection and not by vaccination.

Healthy controls had statistically significant higher Ab titers compared with the entire B-NHL patient cohort (mean titer, 1332 ± 1111 U/mL

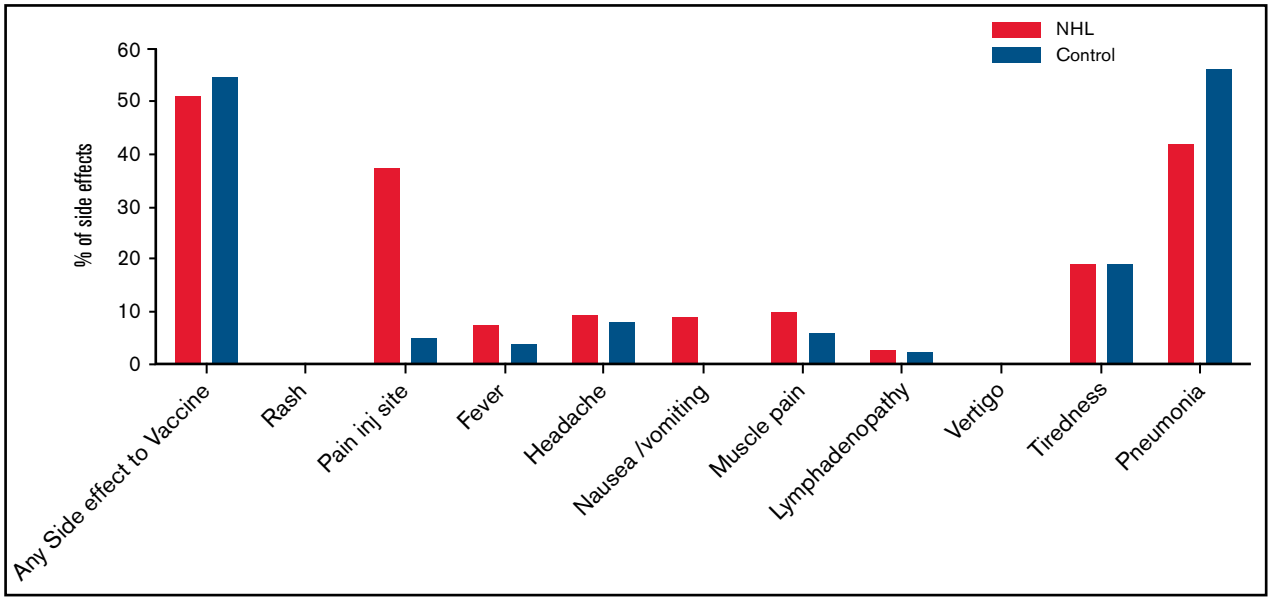

Figure 4. AEs of COVID-19 vaccine in patients with B-NHL vs healthy controls.

vs 440 ± 1124 U/mL, respectively; $P < .001$), as well as when compared with each group of patients, separately (mean 1008 ± 1345 U/mL, 13.7 ± 98.5 U/mL, and 555 ± 1347 U/mL, in patients who were treatment-naïve, actively treated, or >6 months from last anti-CD20 Ab, respectively; $P < .001$). Figure 1B-D presents serology titer levels and distribution in patients with B-NHL and in healthy controls. Figure 2B presents Ab titers in each B-NHL patient subgroup.

Treatment-naïve patients had significantly higher titer levels than those who completed therapy >6 months before vaccination ($P = .034$; Figure 2B).

Univariate analysis of the entire cohort of patients showed treatment status (current R/Obi treatment vs therapy completed >6 months before vaccination vs treatment-naïve; $P < .001$), ALC $\leq 0.9 \times 10^3/\mu L$ vs ALC $> 0.9 \times 10^3/\mu L$ ($P = .002$), and any exposure to R/Obi ($P < .001$) since diagnosis to be significantly associated with lower response rates to the COVID-19 vaccine (Table 2). Multivariate analysis, including age, ALC, disease type (i-B-NHL vs a-B-NHL), and prior exposure to anti-CD20 Abs, confirmed that ALC $\leq 0.9 \times 10^3/\mu L$ vs higher ALC counts and any exposure to anti-CD20 therapy were independent predictors of negative serology (Table 3).

Multivariate analysis for factors predicting the response to COVID-19 vaccine, performed in patients exposed to anti-CD20 Abs (n = 121), including age, ALC, disease type (i-B-NHL vs a-B-NHL), and time since the last anti-CD20 Ab treatment, revealed that shorter time since exposure to anti-CD20 Abs and lower ALC were significant predictors of a negative serological response to anti-COVID-19 vaccine (Table 3). Age and disease subtype had no impact on response rates to the vaccine, although diagnosis of a-B-NHL tended to predict positive serology in this group of patients ($P = .08$).

Adverse events

Sixty of 118 evaluable patients (51%) reported adverse events (AEs). The most common local AE, reported in 44 (37.3%) patients was pain at the injection site. The most common systemic AE was tiredness (n = 23; 19.5%), followed by muscle pain (n = 11; 9.3%). Three (2.5%) patients reported transient lymph node enlargement. All AEs were mild, and all resolved spontaneously. There were no statistically significant differences in types and severity of AEs between patients with B-NHL and healthy controls, except for pain at the injection site, which was reported to be more severe by patients with B-NHL. Figure 4 shows local and systemic AEs in patients and control subjects.

Documentation of infection with COVID-19 after vaccination

Within a median follow-up period of 95 (range, 73-112) days from the second COVID-19 vaccine, 1 patient with lymphoma (who had a seronegative response) and 1 healthy control developed COVID-19 infections.

Discussion

Patients with B-NHL who receive B-cell–depleting therapy are considered to be at increased risk of developing severe COVID-19[5,6,16,17] related to impaired priming of Ab responses and resulting in a compromised ability to neutralize viral replication. Moreover, these patients need a longer time to clear SARS-CoV-2 shedding, suggesting that anti-CD20 therapy poses a higher risk of severe and persistent COVID-19 infection in patients with lymphoma.[16,18]

Effective vaccination against SARS-CoV-2 may help protect patients with lymphoma against COVID-19; however, the intrinsic immune deficiency associated with B-NHL, as well as the antilymphoma treatment itself, may hamper responsiveness to vaccination.[19] Data regarding the efficacy of COVID-19 vaccines in patients with newly diagnosed lymphoma or during or shortly after initiation of antilymphoma treatment are not yet available.

According to our data, patients currently treated with an R/Obi-containing induction regimen and those receiving R/Obi maintenance are not likely to develop a humoral response to the BNT162b2

mRNA COVID-19 vaccine. In contrast, treatment-naïve patients with indolent lymphomas and patients who were vaccinated at least 6, and even 9, months after the last dose of anti-CD20 therapy, were more likely to attain a humoral response to the COVID-19 vaccine and could even achieve high Ab titers. Our findings are in line with those of prior studies[20–22] in which R significantly attenuated response to vaccines, aimed at various pathogens (eg, influenza[23] and hepatitis[24]), for at least 6 months after completion of treatment[13,22] and support recommendations to vaccinate patients with B-cell lymphoma with COVID-19 vaccine before therapy or at least 6 months after completing an anti-CD20 Ab–containing therapy.[19]

Response rates in patients treated with R/Obi showed gradual improvement, with increased response rates with each additional month beyond 6 months from treatment, an increase that became significant at 9 months from last anti-CD20 therapy. These results support a study of response rates to influenza vaccine in patients vaccinated within 6 months after treatment with rituximab that reported significantly low to even null seropositivity rates in R-treated patients with lymphoma, compared with those reported in healthy controls.[13] The sluggish response rates detected in our patients beyond 6 months from anti-CD20 Ab treatment coincide with the reported dynamics of B-cell reconstitution after R treatment, showing that repopulation of the peripheral blood by B cells merely starts at 6 months after R treatment, whereas a more profound recovery occurs later.[25-27]

Achievement of high Ab titers in patients receiving influenza or hepatitis B vaccine, was previously linked to attainment of higher protection rates[28] and durable responses.[29] Lately, high Ab titers after COVID-19 infection have been proposed to be essential for the attainment of durable protection from SARS-CoV-2 infection.[30]

We found that not only seropositivity rates, but also Ab titers were low to undetectable in patients with B-NHL receiving active or maintenance anti-CD20 Ab treatment, yet patients vaccinated >6 months from the end of treatment showed a gradual and consistent increase in the antiviral Ab titer, an increase that was in striking correlation with the time from last exposure to anti-CD20 Ab treatment and was notable, irrespective of age.

Qualitative and quantitative defects in both innate and adaptive immune systems have been reported in treatment-naïve patients with i-B-cell lymphoproliferative disorders, as well as an impaired Ab response to influenza vaccine.[31–33] Treatment-naïve patients with i-B-NHL who were included in our study had lower response rates to the COVID-19 vaccine compared with healthy controls (though higher than in treated patients). This finding is in correlation with those of a recently published study showing similar findings in treatment-naïve patients with chronic lymphocytic leukemia,[34] with both studies emphasizing the inherent immune dysfunction in patients with underlying indolent lymphoma.

In patients previously treated with anti-CD20 Abs, the rates of positive serology were decreased, and median time to achieve positive serology was prolonged in patients with i-B-NHL compared with patients with a-B-NHL, a divergence that seemed to grow over time. These differences probably reflect the deeper immune suppression imposed by longer exposure to anti-CD20 Abs in patients with i-B-NHL who are undergoing maintenance treatment. This result may also indicate the pivotal effects of the underlying lymphoma subtype (incurable i-B-NHL, as opposed to the probably cured a-B-NHL) on the ability to develop a protective immune response to vaccines.

Low lymphocyte counts were a significant predictor of reduced response to the vaccine, data that are in line with previous studies that showed that low lymphocyte counts at vaccination were associated with low response rates to influenza vaccine in patients with cancer.[35,36]

Of note, there was only 1 documented case of COVID-19 infection among our vaccinated patients, which occurred in a patient who failed to achieve humoral response to the vaccine.

Arad et al showed that, even though that treatment with rituximab results in loss of humoral response to influenza vaccine, it does not have substantial effects on cellular immune response.[37] Evaluating long-term immune response in rituximab-treated patients who were vaccinated against influenza[37] showed preservation of cellular immune response, including the ability to develop persistent memory T cells, in the absence of residual humoral response.[37,38] Moreover, it was recently reported that, in patients with NHL who were infected with COVID-19, T-cell response was generally preserved in those recently exposed to B-cell–depleting agents.[39] Thus, it is of great interest to further investigate whether the COVID-19 vaccine can evoke a protective T-cell response in patients with B-NHL who have had anti-CD20 therapy, especially in those who fail to attain humoral response.

Our study has several limitations. First, the relatively small number of patients included in the study, the patients' heterogeneity, and the fact that this was a single-center study may hamper making recommendations regarding vaccination of patients with B-cell NHL at various time points of their treatment and drawing conclusions regarding the potential impact of specific therapeutic agents and regimens on response to vaccination. Second, the protective impact of vaccination and its ability to prevent SARS-CoV-2 infection or clinically significant COVID-19 remained unclear, considering the short follow-up period and the relatively low number of patients. Last, this study evaluated the humoral response to COVID-19 vaccine. However, the accumulated data suggest that attainment of cellular immune response is highly significant in protecting against SARS-CoV-2 infection.[40] Our study, focusing on humoral response, did not address this important issue.

In summary, our study showed that although BNT162b2 mRNA COVID-19 induced a humoral response in a substantial number of patients with B-NHL, irrespective of sex or age, the response was impaired in patients receiving active anti-CD20 Ab treatment. In fact, it seems unlikely that humoral response will be obtained within the first 9 months after anti-CD20 therapy. Response rates gradually increase after that point and continue to grow over time.

Patients with B-NHL who have been treated with an anti-CD20 Ab within 2 years before COVID-19 vaccination should be advised to verify serological response to the vaccine. Yet, given the lack of data on T-cell response to the vaccine in this population of patients, strong conclusions regarding the optimal timing for vaccinating patients treated with B-cell–depleting agents or addressing the question of revaccinating patients who fail to attain humoral response (as well as its timing) cannot be drawn.

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Authorship

Contribution: I.A., C.P., and Y.H. designed the research; I.A., E.L., R.B., and C.P. analyzed the data and wrote the manuscript; and I.A., R.B., R.V., A.A., G.S., M.N., N.K.-K., N.B., Y.T., M.M.M., O.B.-K., M.L., C.P., Y.H., E.L., and Y.C.C. performed the research.

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References

1. Toyoshima Y, Nemoto K, Matsumoto S, Nakamura Y, Kiyotani K. SARS-CoV-2 genomic variations associated with mortality rate of COVID-19. *J Hum Genet.* 2020;65(12):1075-1082.
2. Nagy Á, Pongor S, Győrffy B. Different mutations in SARS-CoV-2 associate with severe and mild outcome. *Int J Antimicrob Agents.* 2021;57(2): 106272.
3. Becerra-Flores M, Cardozo T. SARS-CoV-2 viral spike G614 mutation exhibits higher case fatality rate. *Int J Clin Pract.* 2020;74(8):e13525.
4. Wise J. Covid-19: The E484K mutation and the risks it poses. *BMJ.* 2021;372(359):n359.
5. Lee LYW, Cazier JB, Starkey T, et al; UK Coronavirus Cancer Monitoring Project Team. COVID-19 prevalence and mortality in patients with cancer and the effect of primary tumour subtype and patient demographics: a prospective cohort study. *Lancet Oncol.* 2020;21(10):1309-1316.
6. Lamure S, Duléry R, Di Blasi R, et al. Determinants of outcome in Covid-19 hospitalized patients with lymphoma: A retrospective multicentric cohort study. *EClinicalMedicine.* 2020;27:100549.
7. Bazykin GA, Stanevich O, Danilenko D, et al. Emergence of Y453F and Δ69-70HV mutations in a lymphoma patient with long-term COVID-19. Virological; 4 January 2021. . Accessed 14 July 2021.
8. Kemp SA, Collier DA, Datir RP, et al; COVID-19 Genomics UK (COG-UK) Consortium. SARS-CoV-2 evolution during treatment of chronic infection. *Nature.* 2021;592(7853):277-282.
9. Jackson LA, Anderson EJ, Rouphael NG, et al; mRNA-1273 Study Group. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. *N Engl J Med.* 2020;383(20):1920-1931.
10. Walsh EE, Frenck RW Jr, Falsey AR, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *N Engl J Med.* 2020; 383(25):2439-2450.
11. Polack FP, Thomas SJ, Kitchin N, et al; C4591001 Clinical Trial Group. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med.* 2020;383(27):2603-2615.
12. Baden LR, El Sahly HM, Essink B, et al; COVE Study Group. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med.* 2021; 384(5):403-416.
13. Yri OE, Torfoss D, Hungnes O, et al. Rituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 months after treatment. *Blood.* 2011;118(26):6769-6771.
14. Riester E, Findeisen P, Hegel JK, et al. Performance evaluation of the Roche Elecsys Anti-SARS-CoV-2 S immunoassay [published online ahead of print 2 March 2021]. *medRxiv.* doi: 10.1101/2021.03.02.21252203.
15. Oved K, Olmer L, Shemer-Avni Y, et al. Multi-center nationwide comparison of seven serology assays reveals a SARS-CoV-2 non-responding seronegative subpopulation. *EClinicalMedicine.* 2020;29:100651.
16. Ip A, Mato A, Intrator J, et al. COVID-19 Impact on Lymphoma Patients' Clinical Outcomes - an Observational Cohort Study [abstract]. *Blood.* 2020;136(suppl 1):6-7.
17. Mehta P, Porter JC, Chambers RC, Isenberg DA, Reddy V. B-cell depletion with rituximab in the COVID-19 pandemic: where do we stand? *Lancet Rheumatol.* 2020;2(10):e589-e590.
18. Avanzato VA, Matson MJ, Seifert SN, et al. Case Study: Prolonged Infectious SARS-CoV-2 Shedding from an Asymptomatic Immunocompromised Individual with Cancer. *Cell.* 2020;183(7):1901-1912.e9.
19. De Wilde N, Offner F. Vaccine response in patients receiving anti-B-cell targeted therapy. Belg J Hematol. 2018;9(3):113-117.
20. Eisenberg RA, Jawad AF, Boyer J, et al. Rituximab-treated patients have a poor response to influenza vaccination. *J Clin Immunol.* 2013;33(2):388-396.
21. Hua C, Barnetche T, Combe B, Morel J. Effect of methotrexate, anti-tumor necrosis factor α, and rituximab on the immune response to influenza and pneumococcal vaccines in patients with rheumatoid arthritis: a systematic review and meta-analysis. *Arthritis Care Res (Hoboken).* 2014; 66(7):1016-1026.
22. Bingham CO III, Looney RJ, Deodhar A, et al. Immunization responses in rheumatoid arthritis patients treated with rituximab: results from a controlled clinical trial. *Arthritis Rheum.* 2010;62(1):64-74.
23. van Assen S, Holvast A, Benne CA, et al. Humoral responses after influenza vaccination are severely reduced in patients with rheumatoid arthritis treated with rituximab. *Arthritis Rheum.* 2010;62(1):75-81.

24. Avivi I, Zisman-Rozen S, Naor S, et al. Depletion of B cells rejuvenates the peripheral B-cell compartment but is insufficient to restore immune competence in aging. *Aging Cell.* 2019;18(4):e12959.

25. Colucci M, Carsetti R, Cascioli S, et al. B cell reconstitution after rituximab treatment in idiopathic nephrotic syndrome. *J Am Soc Nephrol.* 2016; 27(6):1811-1822.

26. Abulayha AM, Tabal SA, Shawesh EI, et al. Depletion of peripheral blood B cells with Rituximab and phenotype characterization of the recovering population in a patient with follicular lymphoma. *Leuk Res.* 2010;34(3):307-311.

27. Leandro MJ, Cambridge G, Ehrenstein MR, Edwards JCW. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum.* 2006;54(2):613-620.

28. Dunning AJ, DiazGranados CA, Voloshen T, Hu B, Landolfi VA, Talbot HK. Correlates of Protection against Influenza in the Elderly: Results from an Influenza Vaccine Efficacy Trial. *Clin Vaccine Immunol.* 2016;23(3):228-235.

29. Kakisaka K, Sakai A, Yoshida Y, et al. Hepatitis B surface antibody titers at one and two years after hepatitis B virus vaccination in healthy young Japanese adults. *Intern Med.* 2019;58(16):2349-2355.

30. Jin P, Li J, Pan H, Wu Y, Zhu F. Immunological surrogate endpoints of COVID-2019 vaccines: the evidence we have versus the evidence we need. *Signal Transduct Target Ther.* 2021;6(1):48.

31. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. *Blood.* 2015;126(5):573-581.

32. Fozza C, Corda G, Virdis P, et al. Derangement of the T-cell repertoire in patients with B-cell non-Hodgkin's lymphoma. *Eur J Haematol.* 2015;94 (4):298-309.

33. Brydak LB, Machała M, Centkowski P, Warzocha K, Biliński P. Humoral response to hemagglutinin components of influenza vaccine in patients with non-Hodgkin malignant lymphoma. *Vaccine.* 2006;24(44-46):6620-6623.

34. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood.* 2021;137(23):3165–3173.

35. Yen HJ, Chang WH, Liu HC, et al. Outcomes Following Discontinuation of E. coli l-Asparaginase Upon Severe Allergic Reactions in Children With Acute Lymphoblastic Leukemia. *Pediatr Blood Cancer.* 2016;63(4):665-670.

36. Mavinkurve-Groothuis AMC, van der Flier M, Stelma F, van Leer-Buter C, Preijers FW, Hoogerbrugge PM. Absolute lymphocyte count predicts the response to new influenza virus H1N1 vaccination in pediatric cancer patients. *Clin Vaccine Immunol.* 2013;20(1):118-121.

37. Arad U, Tzadok S, Amir S, et al. The cellular immune response to influenza vaccination is preserved in rheumatoid arthritis patients treated with rituximab. *Vaccine.* 2011;29(8):1643-1648.

38. Bonifacius A, Tischer-Zimmermann S, Dragon AC, et al. COVID-19 immune signatures reveal stable antiviral T cell function despite declining humoral responses. *Immunity.* 2021;54(2):340-354.e6.

39. Hueso T, Pouderoux C, Péré H, et al. Convalescent plasma therapy for B-cell-depleted patients with protracted COVID-19. *Blood.* 2020;136(20): 2290-2295.

40. Sewell HF, Agius RM, Kendrick D, Stewart M. Covid-19 vaccines: delivering protective immunity. *BMJ.* 2020;371:m4838.