Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Humoral and cellular responses to mRNA vaccines against SARS-CoV-2 in patients with a history of CD20 B-cell-depleting therapy (RituxiVac): an investigator-initiated, single-centre, open-label study

Matthias B Moor, Franziska Suter-Riniker, Michael P Horn, Daniel Aeberli, Jennifer Amsler, Burkhard Möller, Linet M Njue, Cesare Medri, Anne Angelillo-Scherrer, Luca Borradori, Susanne Radonjic-Hoesli, S Morteza Seyed Jafari , Andrew Chan, Robert Hoepner, Vera Ulrike Bacher, Laila-Yasmin Mani, Joseena Mariam Iype, Cédric Hirzel, Britta Maurer, Daniel Sidler**

Abstract

Background B-cell-depleting therapies increase the risk of morbidity and mortality due to COVID-19. Evidence-based SARS-CoV-2 vaccination strategies for patients on B-cell-depleting therapies are scarce. We aimed to investigate humoral and cell-mediated immune responses to SARS-CoV-2 mRNA-based vaccines in patients receiving CD20-targeted B-cell-depleting agents for autoimmune disease, malignancy, or transplantation.

Methods The RituxiVac study was an investigator-initiated, single-centre, open-label study done at the Bern University Hospital (Bern, Switzerland). Patients with a treatment history of anti-CD20-depleting agents (rituximab or ocrelizumab) and with no previous history of SARS-CoV-2 infection were enrolled between April 26 and June 30, 2021, for analysis of humoral and cell-mediated immune responses (by interferon-γ [IFNγ] release assay) at least 4 weeks after completing vaccination against SARS-CoV-2. Healthy controls without a history of SARS-CoV-2 infection were also enrolled at least 4 weeks after completing vaccination against SARS-CoV-2. All study participants received two doses of either the Pfizer–BioNTech BNT162b2 vaccine or the Moderna mRNA-1273 vaccine. The primary outcome was the proportion of patients with a history of anti-CD20 treatment who showed a humoral immune response against the SARS-CoV-2 spike protein in comparison with immunocompetent controls. Prespecified secondary endpoints were the effect of anti-CD20 therapy (including time since last treatment and cumulative dose) on humoral or cell-mediated immune responses to SARS-CoV-2 vaccination, and biomarkers of immunocompetence. This study is registered with ClinicalTrials.gov, NCT04877496.

Findings The final study population comprised 96 patients and 29 immunocompetent controls. The median age of patients was 67 years (IQR 57–72) and of controls was 54 years (45–62), and 51 (53%) of 96 patients and 19 (66%) of 29 controls were female. The median time since last anti-CD20 treatment was 1·07 years (IQR 0·48–2·55) and the median cumulative dose of an anti-CD20 depleting agent was 2·80 g (1·50–5·00). Anti-spike IgG antibodies were detected in 47 (49%) of 96 patients 1·79 months (IQR 1·16–2·48) after the second vaccine dose compared to 29 (100%) of 29 controls 1·81 months (1·17–2·48) after the second vaccine dose (p<0·001). SARS-CoV-2-specific IFNγ release was detected in 14 (32%) of 44 patients and 22 (88%) of 25 healthy controls (p<0·001). Only ten (23%) of 44 patients were double positive for anti-SARS-CoV-2 spike IgG and cell-mediated responses, compared with 22 (88%) of 25 healthy controls (p<0·001). Time since last anti-CD20 therapy (>7·6 months; positive predictive value 0·78), peripheral CD19⁺ cell count (>27 cells per µL; positive predictive value 0·70), and CD4⁺ lymphocyte count (>653 cells per µL; positive predictive value 0·71) were predictive of humoral vaccine response (area under the curve [AUC] 67% [95% CI 56–78] for time since last anti-CD20 therapy, 67% [55–80] for peripheral CD19⁺ count, and 66% [54–79] for CD4⁺ count).

Interpretation This study provides further evidence of blunted humoral and cell-mediated immune responses elicited by SARS-CoV-2 mRNA vaccines in patients with a history of CD20 B-cell-depleting treatment. Lymphocyte subpopulation counts were associated with vaccine response in this highly vulnerable population. On validation, these results could help guide both the administration of SARS-CoV-2 vaccines and B-cell-depleting agents in this population.

Funding Bern University Hospital.

Introduction

The COVID-19 pandemic has taken a toll on many patients worldwide. Age and male sex are important drivers for severe COVID-19 trajectories, as are pre-existing autoimmune or kidney disease and malignancy.[1–3] The backbone of pandemic-ending strategies is mass vaccination.[4,5] Although randomised controlled trials of mRNA-based vaccines reported high vaccine efficacy,[6]

Lancet Rheumatol 2021; 3: e789–97

This online publication has been corrected. The corrected version first appeared at thelancet.com/rheumatology on January 20, 2022

See **Comment** page e741

*Contributed equally

Department of Nephrology and Hypertension (M B Moor MD-PhD, L-Y Mani MD, D Sidler MD-PhD), **Department of Clinical Chemistry** (M P Horn PhD, J M Iype PhD), **Department of Rheumatology and Immunology** (D Aeberli MD, J Amsler MD, B Möller MD, B Maurer MD), **Department of Haematology and Central Haematology Laboratory** (L M Njue MD, C Medri MMed, A Angelillo-Scherrer MD, V Ulrike Bacher MD), **Department of Dermatology** (L Borradori MD, S Radonjic-Hoesli MD-PhD, S M Seyed Jafari MD-PhD), **Department of Neurology** (A Chan MD, R Hoepner MD-PhD), **and Department of Infectious Diseases** (C Hirzel MD), **Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; Department of Biomedical Research** (M B Moor, L-Y Mani, D Sidler, D Aeberli, J Amsler, B Möller, B Maurer) **and Institute for Infectious Diseases** (F Suter-Riniker PhD), **University of Bern, Bern, Switzerland**

Correspondence to:
Dr Daniel Sidler, Department of Nephrology and Hypertension, Inselspital, Bern University Hospital, University of Bern, 3010 Bern, Switzerland
daniel.sidler@insel.ch

Research in context

Evidence before this study

We searched Medline, PubMed, and the medRxiv preprint server on Feb 26, 2021, using the search terms ("COVID vaccine rituximab"), ("SARS-CoV-2 vaccination rituximab") AND ("COVID-19 vaccination rituximab"). No date or language restrictions were applied. We identified no studies assessing humoral or cellular immune responses of SARS-CoV-2 mRNA vaccines in patients receiving B-cell-depleting drugs. However, editorials and commentaries have suggested an increased risk of impaired immune responses in this patient population, on the basis of studies of conventional vaccines against other viral or bacterial pathogens before the COVID-19 pandemic. We re-ran the search on Aug 19, 2021, and found a series of studies that investigated humoral and cellular responses to SARS-CoV-2 vaccination in patients with a history of B-cell depleting anti-CD20 therapies. Briefly, these studies found impaired humoral and cellular responses after two doses of SARS-CoV-2 vaccination in this population.

Added value of this study

In this study, we showed that patients taking B-cell-depleting therapies were able to mount responses to SARS-CoV-2 vaccination only if certain prerequisites were met. We identified timing of anti-CD20 therapy, immunosuppressive co-medication, and peripheral B-cell and T-cell status as determining factors. The length of the post-treatment interval and $CD19^+$ and $CD4^+$ cell counts might also have potential as predictive markers as they reliably identified vaccination responders. Additionally, our data support the importance of $CD4^+$ T cells in providing protection against SARS-CoV-2 infection, since both humoral and cellular-mediated immune responses were dependent on $CD4^+$ T-cell numbers.

Implications of all the available evidence

Vaccination strategies exclusively based on a rigid CD20-depletion interval or peripheral B-cell count would render many patients ineligible for SARS-CoV-2 vaccination, especially the most vulnerable patients undergoing the most aggressive treatment regimens and at greatest risk of developing severe COVID-19. If the predictive potential of $CD4^+$ T-helper-cell counts as an immune biomarker for SARS-CoV-2 mRNA-based vaccines is confirmed in future prospective studies, individually tailored but easily applicable vaccination strategies could be derived. This approach could also inform policies and guidelines for the use of other recommended vaccines in immunocompromised patients.

these trials did not include immunocompromised patients, who can be expected to have inferior responses to vaccination. The likelihood of impaired responses is particularly high for patients treated with B-cell-depleting agents.[7] Anti-CD20 B-cell therapies are administered to patients worldwide, with annual doses ranging in the millions.[8] The B-cell-depleting drug rituximab or biosimilar agents are serially administered for a broad spectrum of autoimmune and alloimmune disorders and haematological neoplasms. Patients treated with B-cell-depleting agents have been shown to be particularly vulnerable to COVID-19, having four times higher odds of COVID-19-related mortality compared with patients on other immunosuppressive medication such as methotrexate.[9] The high variability of pharmacokinetics and thereby B-cell recovery times makes it difficult to define ideal vaccination timepoints and to predict immune responses to vaccines.[10] There is an urgent need for evidence-based recommendations for administration of SARS-CoV-2 vaccines in immunocompromised patients, since in the absence of randomised controlled trials, current recommendations to delay B-cell-depleting therapies are based on previous influenza vaccination studies[7] and emerging humoral data on immune responses to SARS-CoV-2 vaccines in immunocompromised patients.[11–16] An improved understanding of humoral and cell-mediated responses following vaccination against SARS-CoV-2 in patients treated with anti-CD20-depleting agents is a prerequisite for the development of individualised vaccination strategies for this population. Notably, longitudinal observational data after SARS-CoV-2 infection and after mRNA vaccine administration has provided evidence that, in COVID-19 and after vaccination, cell-mediated immune responses were crucial for humoral immunity[17–21] and might provide protection even in patients with B-cell depletion.[22]

The absence of evidence-based vaccination strategies in this highly vulnerable population prompted the RituxiVac study, which aimed to assess both humoral and cell-mediated immune responses to SARS-CoV-2 mRNA-based vaccines in patients with a history of anti-CD20 therapy.

Methods

Study design and participants

The RituxiVac study was an investigator-initiated, single-centre, open-label trial done at the Departments of Nephrology and Hypertension, Rheumatology and Immunology, Haematology, Neurology, and Dermatology at the Bern University Hospital (Bern, Switzerland). The trial design and study protocol are provided in the appendix (pp 15–18). Briefly, COVID-19-naive patients with a history of anti-CD20 therapy (rituximab or ocrelizumab) and completion of SARS-CoV-2 vaccination for 4 weeks or longer were enrolled between April 26 and June 30, 2021. Data on treatment type, timepoints, cumulative dose, and treatment indication for anti-CD20 therapies were recorded. All treatments taken since Jan 1, 2010, until the date of the first dose of the SARS-CoV-2 mRNA vaccine

See Online for appendix

were considered. Additionally, data on patients' age, sex, and immunosuppressive co-medications were recorded. Information about vaccine type and date of administration was derived from official records and COVID-19 vaccination certificates provided by participants. Additionally, healthy controls without a history of SARS-CoV-2 infection were enrolled among the personal and professional contacts of the study investigators. No stringent matching was done between patients and healthy controls. All controls also received two doses of their respective SARS-CoV-2 vaccine at least 4 weeks before the study visit.

Patients and healthy controls with previous SARS-CoV-2 infection were not eligible for inclusion in the study. All participants were tested for the presence of anti-nucleocapsid antibodies, and individuals with positive results were excluded from the analysis. Individuals younger than 18 years and pregnant or lactating women were not eligible for inclusion.

In Switzerland, SARS-CoV-2 vaccines were administered on the basis of an age-tailored and risk-tailored national priority plan. The Pfizer–BioNTech mRNA vaccine (BNT162b2; also known as tozinameran) was approved on Dec 19, 2020; the Moderna SARS-CoV-2 vaccine (mRNA-1273; also known as elasomeran) was approved on Jan 12, 2021. All participants received two doses of either the BNT162b2 vaccine or the mRNA-1273 vaccine. The allocation, administration, and reporting of vaccination was coordinated and supervised by Swiss federal authorities independently of the study protocol.

The trial protocol was approved by the local ethics committee of the Canton of Bern, Switzerland (ID 2021–00669), and is registered on ClinicalTrials.gov (NCT04877496). The trial was done in accordance with the principles of the Declaration of Helsinki, and all participants provided written informed consent before inclusion in the study.

Procedures

Data on disease, treatment, and vaccination history were obtained by trained study nurses and physicians with a 17-item questionnaire. Where available, information about dates and types of administered vaccines (BNT162b2 or mRNA-1273) was obtained from official vaccination records.

Blood was collected in lithium heparin tubes (for the interferon-γ [IFNγ] release assay) and serum tubes (for antibody measurements and analysis of lymphocyte subpopulations). Serum tubes were centrifuged, and serum was then aliquoted and stored at –20°C before analyses. Creatinine values, lymphocyte subpopulation counts, and total immunoglobulin quantities (IgG, IgM, and IgA) were obtained at the post-vaccination study visit as part of the routine clinical analytical services of the Centre of Laboratory Medicine, Department of Clinical Chemistry, at the University Hospital Bern.

To assess humoral responses to vaccines, IgG antibodies targeting the SARS-CoV-2 spike (S1) protein were detected with a commercial ELISA test from Euroimmun (Lübeck, Germany), as previously described.[23] In brief, samples were diluted 1:100 in sample buffer. For antibody binding, 100 µL of diluted samples, prediluted positive and negative controls, and a prediluted calibrator were added for 1 h at 37°C. After three washing steps, 100 µL of horseradish peroxidase (HRP)-labelled secondary anti-human IgG antibodies was added for 30 min at 37°C, followed by three more washing steps. Finally, 100 µL of 3,3' ,5,5' -tetramethylbenzidine (TMB) solution was added for 20 min. The reaction was stopped with 100 µL of 0·5M H_2SO_4, and results were measured at an optical density (OD) of 450–620 nm. Antibody values were expressed as a ratio of the OD of the sample to the OD of the calibrator. All samples with a ratio higher than 1·1 were considered as positive as per the manufacturer's instructions. To exclude participants with previous COVID-19, an anti-nucleocapsid enhanced chemiluminescence immunoassay (ECLIA) test was done on a Cobas 8000 analyser (Roche Diagnostics, Rotkreuz, Switzerland).[24] The cutoff was calculated on the basis of the calibrator measurements and an index signal to cutoff ratio of 1·0 or higher was considered positive for nucleocapsid IgG, as per the manufacturer's instructions. A humoral response was defined as an anti-SARS-CoV-2 spike (S1) of 1·1 or higher (index).[25]

To assess cell-mediated immune responses to vaccination, SARS-CoV-2-specific IFNγ release in whole blood was measured in 64 participants (44 patients and 25 healthy controls) by use of the QuantiFERON SARS-CoV-2 Starter Pack (Qiagen, Hombrechtikon, Switzerland; category number 626715), which contains two different pools of the protein S peptide. Following manufacturer's instructions, whole blood was incubated with peptide pools or mitogen for 16 h. Subsequently, IFNγ was quantified by ELISA (Qiagen category number 626410). All samples showed a positive response to mitogen. Responses to antigen pool 1 were analysed. A cutoff value of 0·15 IU/mL was used to discriminate positive from negative cell-mediated immune responses to SARS-CoV-2, as reported previously.[26]

Outcomes

The primary outcome was the proportion of patients with a history of anti-CD20 treatment who showed a humoral immune response against the SARS-CoV-2 spike protein at least 4 weeks after completion of SARS-CoV-2 vaccination in comparison with immuno-competent controls.

Prespecified secondary endpoints were the effect of anti-CD20 therapy, including time since last treatment and cumulative dose, on humoral or cell-mediated immune responses to SARS-CoV-2 mRNA-based vaccines in linear regression models adjusted for vaccine type, age, sex, immunosuppressive co-medication, and blood markers of immunocompetence

(concentrations of IgG, IgM, and IgA, absolute lymphocyte counts, and absolute CD19, CD3, and CD4 cell counts). After following the prespecified data analysis, we assessed the discriminative power of selected biomarkers by calculating the area under the receiver operating characteristic curve (AUC-ROC) to predict vaccine-elicited humoral and cell-mediated immune responses.

	Patients (n=96)	Healthy controls (n=29)	p value
Sex			
Female	51 (53%)	19 (66%)	..
Male	45 (47%)	10 (34%)	0·20
Median age, years	67 (57–72)	54 (45–62)	<0·001
Immunosuppression	57 (59%)	0 (0%)	<0·001
Pfizer–BioNTech BNT162b2 vaccine	58 (60%)	9 (31%)	0·0050
Moderna mRNA-1273 vaccine	38 (40%)	20 (69%)	..
Median time since vaccination, months	1·79 (1·16–2·48)	1·81 (1·17–2·48)	0·20
Median time since last anti-CD20 therapy, years	1·07 (0·48–2·55)	..	..
Median cumulative dose of anti-CD20 therapy, g	2·80 (1·50–5·00)	..	..
Immunosuppressive co-medication (n=57)			
Prednisolone	45/57 (79%)	..	..
Calcineurin inhibitors	19/57 (33%)	..	..
Antimetabolites	24/57 (42%)	..	..
Methothrexate	4/57 (7%)	..	..
Biologic	1/57 (2%)	..	..
Chemotherapy	4/57 (7%)	..	..
Other immunosuppression	2/57 (4%)*	..	..
Disease			
ANCA-associated vasculitis	20 (21%)	..	..
ABO-incompatible transplantation	19 (20%)	..	..
B-cell lymphoma	6 (6%)	..	..
Rheumatoid arthritis	6 (6%)	..	..
Sjögren's syndrome	6 (6%)	..	..
Systemic lupus erythematosus	6 (6%)	..	..
Autoimmune haemolytic anaemia	5 (5%)	..	..
Multiple sclerosis	5 (5%)	..	..
IgG4-associated disease	4 (4%)	..	..
Membranous nephropathy	4 (4%)	..	..
Pemphigus vulgaris	4 (4%)	..	..
Systemic sclerosis	4 (4%)	..	..
Immune-mediated necrotising myopathy	3 (3%)	..	..
Pemphigoid	3 (3%)	..	..
Overlap syndrome	2 (2%)	..	..
Focal segmental glomerulosclerosis	1 (1%)	..	..
IgA-associated vasculitis	1 (1%)	..	..
Minimal change disease	1 (1%)	..	..
Thrombotic thrombocytopenic purpura	1 (1%)	..	..

Data are n (%) or median (IQR). Immunosuppression refers any of the following: prednisolone, calcineurin inhibitors, antimetabolites, methotrexate, cytotoxic chemotherapy or immunosuppressive or immunomodulatory biologics (apart from anti-CD20 therapy). p values computed by Pearson's r^2 test and Wilcoxon rank sum test. ANCA=antineutrophil cytoplasmic antibody. *One patient received intravenous immunoglobulins and one received an mTOR inhibitor.

Table 1: **Baseline characteristics, vaccination history of patients and healthy controls, and anti-CD20 B-cell depletion history of patients in the study**

Statistical analysis

Pre-screening revealed an eligible population of 725 participants with a history of taking at least one anti-CD20 treatment since Jan 1, 2010. With the assumption that 70% of patients and 98% of healthy controls would reach the dichotomous outcome of a humoral response, a minimal sample size of 18 healthy controls and 72 patients was determined, with an enrolment ratio of 4:1 (two-sided test, alpha error of 0·05, beta error of 0·8).

Statistical analyses were done with R, version 4.0.4. A r^2 test was done to compare categorical variables between two groups. The Mann-Whitney *U* test or *t*-test was done to compare continuous variables between groups, as appropriate. Linear regression analyses were done according to a statistical analysis plan with the lm function and logistic regression with the glm function in R. Selected regression models were visualised with the R package visreg.[27] AUC-ROCs were computed with the R package pROC. Statistical significance was determined at p values less than 0·05; p values and widths of 95% CIs were not adjusted for multiplicity.

Role of the funding source

The study was supported by internal institutional grants of the investigators; the funder had no influence on the design or conduct of the trial and was not involved in data collection or analysis, in the writing of the manuscript, or in the decision to submit the manuscript for publication.

Results

Between April 26 and June 30, 2021, 106 patients and 30 healthy controls were enrolled (appendix p 2). After exclusion of six participants (five patients and one healthy control) who had positive anti-nucleocapsid antibodies and five patients who received the first dose of rituximab after the first vaccination, the final study population comprised 96 patients and 29 healthy controls. Anti-CD20 therapies were prescribed for autoimmune disease in 71 (74%) patients, for malignancy in six (6%) patients, and for induction therapy of ABO-incompatible kidney transplantation in 19 (20%) patients. Demographic details, treatment history, vaccination data, and details of indications leading to rituximab (93 [97%] patients) or ocrelizumab (three [3%] patients) treatment are in table 1. 57 (59%) patients reported immunosuppressive co-medication, among them corticosteroids in 45 (79%) of 57 cases, calcineurin inhibitors in 19 (33%) cases, antimetabolites in 24 (42%) cases, methotrexate in four (7%) cases, cytotoxic chemotherapy in four (7%) cases, or other immunosuppressive drugs in two (4%) cases (one received intravenous immunoglobulins and one received an mTOR inhibitor). Data on ethnicity were

not collected; however, the majority of patients and all volunteers were of white ethnicity. None of the healthy controls received treatment with immunosuppressive agents or anti-CD20 therapy.

Patients were more frequently vaccinated with the BNT162b2 vaccine than controls (table 1). The median time since last anti-CD20 treatment was 1·07 years (IQR 0·48–2·55). 26 (27%) of 96 patients received vaccination within 6 months, 47 (49%) patients within 12 months and 65 (68%) patients within 24 months after last anti-CD20 therapy. The median cumulative dose of an anti-CD20 depleting agent was 2·80 g (IQR 1·50–5·00).

Baseline markers are provided in table 2. Patients had significantly lower peripheral CD3, CD4, and CD19 cell counts than healthy controls (p<0·001). Absolute IgG and IgA concentrations were similar, yet patients had moderately reduced IgM concentrations (p<0·001).

We identified a median spike (S1) IgG concentration of 7·34 (IQR 6·44–8·00) index signal to cutoff ratio in healthy controls and 0·74 (0·13–5·75) in patients with a history of anti-CD20 therapy (p<0·001; appendix p 4). Anti-spike IgG antibodies above the cutoff were present in 29 (100%) of 29 healthy controls and 47 (49%) of 96 patients (p<0·001; table 3; data disaggregated by sex are in the appendix p 11). When stratified for treatment indication, 42 (59%) of 71 patients treated for autoimmunity were positive for anti-spike IgG antibodies, as were three (50%) of six patients treated for cancer, and two (11%) of 19 for ABO-incompatible transplantation (appendix p 5). Cell-mediated immune responses to SARS-CoV-2 were significantly different among the groups, with a median SARS-CoV-2-specific IFNγ release of 0·62 UI/mL (IQR 0·27–1·01) in healthy controls and 0·04 (0·01–0·21) in patients (p<0·001; appendix p 4). Therefore, 22 (88%) of 25 healthy controls and 14 (32%) of 44 patients had responses above the cutoff (>0·15 IU/mL; p<0·001). Overall, 22 (88%) of 25 healthy controls were double positive for anti-SARS-CoV-2 spike IgG and cell-mediated responses, compared with ten (23%) of 44 patients. 20 (45%) of 44 patients were double negative for anti-SARS-CoV-2 anti-spike IgG and cell-mediated responses compared with no healthy controls.

Next, to establish predictors for successful vaccination strategies in patients who received anti-CD20 therapy, we analysed demographics, medical history, and biomarkers in linear regression models. In univariable models, age had no effect on humoral responses in both patients and in healthy controls (figure A). However, peripheral CD19⁺ B-cell count (figure B), total serum IgM concentrations (figure C), time (in years) since last anti-CD20 treatment (figure D), and CD4⁺ T-cell helper count (figure E) showed positive associations with circulating anti-spike antibodies. Total serum IgG concentrations were not associated with humoral vaccine responses (figure F). In the multivariable linear regression analysis, cumulative dose of anti-CD20 therapy and time (in years) since last treatment were independent predictors of vaccine-elicited humoral immune responses (p<0·001; table 4; data disaggregated by sex are in the appendix [pp 12–13]). Furthermore, the mRNA-1273 vaccine elicited more pronounced responses than the BNT162b2 vaccine (p=0·0050), while concomitant immunosuppressive medication independently blunted responses (p<0·001). Peripheral CD4⁺ T-helper-cell counts, CD19⁺ cell counts, and IgM concentrations independently predicted vaccination antibody response. Peripheral CD19⁺ cell counts and co-existing immunosuppressive medication were the only determinants that affected vaccine-elicited cell-mediated immune responses (p<0·001). Taken together, these analyses highlight the importance of anti-CD20 therapy timing and peripheral CD4⁺ and CD19⁺ lymphocyte counts for immune responses to vaccines. To explore these interactions further, we plotted anti-SARS-CoV-2 IgG concentrations against CD19 cell numbers and time since CD20 depletion for various levels of CD4 counts. CD4 cells positively correlated with IgG responses, notably in settings of low CD19 counts or short intervals since CD20 B-cell depletion (figure G–H).

We then applied ROC curves to evaluate the classification performance of the three most promising clinical and laboratory characteristics to predict the humoral response to SARS-CoV-2 vaccines. IgG concentrations and CD19⁺ and CD4⁺ cell counts are

	Patients (n=96)	Healthy controls (n=29)	p value	Reference*
Lymphocytes (cells per µL)	1344 (895–1720)	2275 (2061–2368)	<0·001	1200–2800
CD3 cells (per µL)	1016 (662–1357)	1670 (1312–1756)	<0·001	690–2540
CD4 cells (per µL)	658 (459–958)	1061 (958–1257)	<0·001	410–1590
CD19 cells (per µL)	9 (1–84)	236 (228–290)	<0·001	90–660
IgG (g/L)	8 (7–10)	10 (8–10)	0·14	7·0–16·0
IgA (g/L)	1·62 (1·08–2·41)	1·74 (1·39–2·34)	0·50	0·7–4·0
IgM (g/L)	0·48 (0·30–0·73)	0·80 (0·69–1·22)	<0·001	0·4–2·3

Data are median (IQR). CD=cluster of differentiation. p values computed by Wilcoxon rank sum test. *Normal reference values.

Table 2: **Laboratory markers of immune competence in peripheral blood of patients and healthy controls at time of study visit**

	Patients (n=96)	Healthy controls (n=29)	p value
Anti-SARS-CoV-2 S1 IgG (>1·1 index)	47/96 (49%)	29/29 (100%)	<0·001
IFNγ release (>0·15 IU/mL)	14/44 (32%)	22/25 (88%)	<0·001
S1 IgG positive and IFNγ positive	10/44 (23%)	22/25 (88%)	<0·001
S1 IgG negative and IFNγ negative	20/44 (45%)	0/25 (0%)	<0·001

Data are n/N (%). Frequency of anti-SARS-CoV-2 S1 IgG response above a threshold of 1·1 (index signal to cutoff ratio), IFNγ release higher than 0·15 IU/mL for patients and healthy controls. p values computed by Pearson's r^2 test. IFNγ=interferon-γ.

Table 3: **Frequency of positive humoral and cellular anti-SARS-CoV-2 responses**

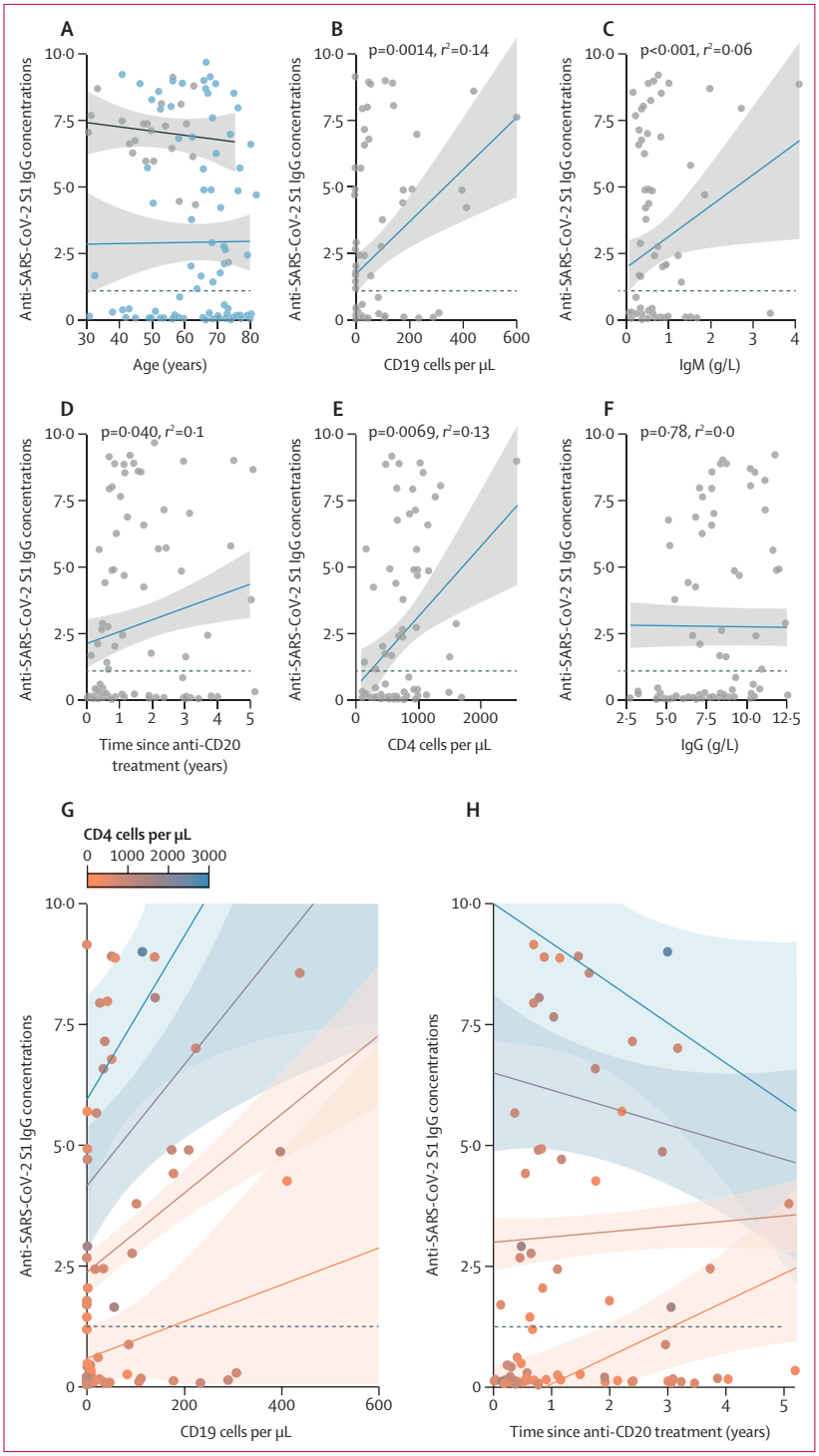

Figure: **Univariate correlation between anti-SARS-CoV-2 spike (S1) IgG concentrations and clinical and serological variables of immunocompetence**

(A) Linear regression between anti-SARS-CoV-2 spike (S1) IgG concentrations and participants' age. (B–F) Linear regression between anti-SARS-CoV-2 S1 IgG concentrations and indicated variables. The shaded grey area represents the 95% CI for the regression line. Each grey datapoint represents one individual. r^2represents the regression coefficient. The dotted line denotes the cut-off anti-SARS-CoV-2 S1 IgG value of 1·1 (signal to cutoff ratio). (G) Linear regression between anti-SARS-CoV-2 S1 IgG concentrations and CD19 cell count. (H) Time (in years) since CD20 depletion for various levels of CD4 cell count. Each datapoint represents one patient.

surrogate markers for immune competence. The sensitivity and specificity of time (in years) since last treatment, peripheral CD19⁺ counts, and CD4⁺ counts to predict a dichotomous anti-SARS-CoV-2 humoral response are shown in the appendix (p 3). Time since last anti-CD20 therapy, peripheral CD19⁺ cell count and CD4⁺ lymphocyte count were predictive of humoral vaccine response (AUC 67% [95% CI 56–78] for time since last anti-CD20 therapy, 67% [55–80] for peripheral CD19⁺ count, and 66% [54–79] for CD4⁺ count). Analyses revealed optimal cutoffs at more than 7·6 months since the last treatment (positive predictive value 0·78), more than 27 CD19⁺ cells per µL (positive predictive value 0·70), and more than 653 CD4⁺ cells per µL (positive predictive value 0·71).

We also analysed peripheral immune cell counts and vaccine responses depending on B-cell depletion status, presence of co-immunosuppressive therapy, and time since last anti-CD20 treatment. Patients with normal peripheral CD19⁺ cell counts (>90 cells per µL) had higher CD4⁺ cell counts and both humoral and cellular immune responses to vaccination than did patients who showed low (1–89 cells per µL) or even undetectable levels of peripheral CD19⁺ cells at the post-vaccination study visit (appendix p 6). Similarly, patients who had received anti-CD20 monotherapy showed higher peripheral CD4⁺ cell counts and both humoral and cellular immune responses compared with patients who were co-treated with another immunosuppressive therapy (appendix p 7). Finally, CD19⁺ B cell counts increased with a longer delay since last treatment: At less than 6 months after anti-CD20 treatment, two (9%) of 22 patients fulfilled the threshold of 27 CD19⁺ B cells per µL, compared with eight (20%) of 41 patients less than 12 months after anti-CD20 treatment, and 24 (62%) of 39 patients 12 months or longer after anti-CD20 treatment, which is suggestive of a favourable vaccination outcome. Meanwhile, 11 (50%) of 22 patients met the criterion of at least 653 CD4 cells per µL (which has a similar predictive value for successful SARS-CoV-2 vaccination) less than 6 months after anti-CD20 treatment, as did 18 (44%) of 41 patients less than 12 months after anti-CD20 treatment, and 22 (56%) of 39 patients 12 months or longer after anti-CD20 treatment (appendix p 8). Humoral responses were low in the first 6 months after anti-CD20 therapy and increased thereafter. Meanwhile, cell-mediated immune responses were stable for all patient groups, independently of time since anti-CD20 treatment. In the subgroup of patients with less than 6 months between anti-CD20 therapy and SARS-CoV-2 vaccination, a cutoff of 653 CD4 cells per µl/L had a positive predictive value of 0·9.

Discussion

The results of this investigator-initiated, single-centre, open-label trial show that the interval since last CD20 depletion and numbers of circulatory CD19 or CD4 cells, or both, predict SARS-CoV-2 vaccination response in

patients with a history of anti-CD20 therapy. These variables could thus be used to optimise and individualise vaccination strategies.

Two factors made the present study well suited to evaluating the immune responses to SARS-CoV-2 mRNA-based vaccines in a real-life setting. First, the combination of detailed clinical and laboratory background data provided by the integrative data repository centre of our tertiary referral centre at the Bern University Hospital; and second, the rapid pace of the vaccination campaign with a correspondingly short post-vaccination follow-up period.

Rituximab and biosimilars are crucial backbones in the treatment of patients with autoimmune disorders or B-cell mediated malignancy, or both. A sufficiently frequent anti-CD20 dosing strategy and suppression of peripheral B cells are treatment goals in such patients, especially those with active or progressive disease. Therefore, a tailored vaccination strategy based on the CD20 depletion interval or peripheral B-cell count would deem many patients ineligible for vaccination, especially those on the most aggressive treatment regimens, who are also at greatest risk for severe COVID-19 trajectories.

Our report adds to the results of a series of studies that have investigated humoral and cellular responses to SARS-CoV-2 vaccination in patients with a history of B-cell depleting anti-CD20 therapies.[11,12,16,28–31] Deepak and colleagues[11] first reported a 36-fold decrease in vaccine-induced anti-spike IgG titres in patients with B-cell depleting therapies. Simon and colleagues[30] and Apostolidis and colleagues[29] reported no detectable or severely diminished vaccine-induced humoral immune responses compared with controls in two smaller series of patients with severely depleted B-cell counts, but both studies reported detectable cell-mediated immunity in these patients. In our study, humoral responses against SARS-CoV-2-specific mRNA-based vaccines were observed in all healthy controls but in only in a subset of patients on anti-CD20 therapy. When stratified for treatment indication, patients with autoimmune diseases had a higher response rate than those who underwent transplantation or patients with cancer, which might arise from differences in concomitant immunosuppressive treatment. Similarly, vaccine-elicited cellular responses were much higher in controls than in patients. Overall, whereas the majority of healthy individuals mounted successful humoral and cellular vaccination responses, this only applied to a few patients who received anti-CD20 therapy, thereby underlining the complex sequelae of B-cell depletion on B-cell and T-cell interactions. In addition to the expected lower CD19$^+$ B-cell counts and IgM concentrations, patients had decreased numbers of CD3$^+$ and CD4$^+$ T cells. These findings support the notion that selective B-cell depletion indirectly results in a reduction of certain subsets of T lymphocytes,[32,33] which might further impair vaccination efficacy.

	Beta coefficient for anti-SARS-CoV-2 spike IgG		Beta coefficient for IFNγ release	
	β (95% CI)	p value	β (95% CI)	p value
Models for laboratory variables				
Total lymphocytes (per 1000/µL)	–1·10 (–3·20 to 0·88)	0·26	0·02 (–0·14 to 0·09)	0·70
CD4 cells (per 1000/µL)	4·50 (1·30 to 7·70)	0·0070	0·05 (–0·16 to 0·27)	0·61
CD19 cells (per 1000/µL)	10·00 (4·60 to 15·00)	<0·001	0·88 (0·41 to 1·30)	<0·001
IgG (per g/L)	–0·05 (–0·12 to 0·02)	0·13	0·02 (0·00 to 0·04)	0·067
IgA (per g/L)	0·24 (–0·34 to 0·81)	0·41	–0·11 (–0·19 to –0·03)	0·013
IgM (per g/L)	1·40 (0·49 to 2·20)	0·0030	–0·06 (–0·13 to 0·02)	0·13
Models for clinical variables				
Male sex	–0·23 (–1·40 to 0·94)	0·69	–0·19 (–0·45 to 0·08)	0·16
Age per year	–0·01 (–0·06 to 0·03)	0·55	–0·01 (–0·02 to 0·00)	0·10
Cumulative anti-CD20 dose (per g)	0·33 (0·14 to 0·52)	<0·001	–0·01 (–0·05 to 0·03)	0·66
Time since anti-CD20 therapy (per year)	0·55 (0·24 to 0·86)	<0·001	–0·02 (–0·08 to 0·05)	0·62
Immunosuppressive medication	–2·10 (–3·30 to –0·93)	<0·001	–0·32 (–0·56 to –0·08)	0·010
Vaccine type (Moderna mRNA-1273)	1·70 (0·53 to 2·90)	0·0050	0·12 (–0·13 to 0·38)	0·34

Individual multivariable linear regression models were computed, with humoral or cellular immune response as dependent variables and sets of clinical or laboratory variables important for immune competence in patients with a history of anti-CD20 therapy as independent variables. Interactions between the determinants were analysed and given when a p value less than 0·05 was reached.

Table 4: **Multivariable linear regression analysis for humoral and cellular anti-SARS-CoV-2 responses**

Strengths of this study include the identification of potential predictors for vaccination efficacy in patients on anti-CD20 therapy. For adequate humoral responses, the timing of anti-CD20 therapy, CD19$^+$ counts, and IgM concentrations were crucial. CD4$^+$ counts positively predicted adequate humoral. These results, as well as the observed positive correlation of CD4$^+$ cell counts with anti-spike IgG antibodies, indicate an important role of T cells for vaccination efficacy in B-cell-depleted patients, as recently suggested.[30] Notably, although the mRNA-1273 and BNT162b2 vaccines are very similar, apart from differences in the structure of lipid nanoparticles,[34] the mRNA-1273 vaccine was associated with more pronounced humoral and cellular responses. Given the comparatively lower number of individuals who received the mRNA-1273 vaccine, which was approved later, this finding should be interpreted with caution. Another noteworthy observation was the fact that immunosuppressive co-medication impaired both humoral and cellular vaccine-elicited immune responses, which is an important consideration for the individualisation of vaccination strategies. Cutoff points for CD4$^+$ and CD19$^+$ counts defined by the ROC analysis allowed vaccination responders to be distinguished from non-responders at a given time interval after the last anti-CD20 therapy. This finding could guide future

studies directly addressing biomarkers of vaccination responses in larger populations.

The present study had a number of limitations. First, we could not measure anti-SARS-CoV-2 spike protein antibodies before vaccination, because the Swiss vaccination programme was accelerated in high-risk individuals before recruitment was initiated. Therefore, we measured currently circulating anti-SARS-CoV-2 nucleocapsid antibodies and excluded the three participants with detectable anti-nucleocapsid antibodies. Second, the distribution of vaccines manufactured by Pfizer–BioNTech and Moderna was not identical between healthy controls and patients. The BNT162b2 vaccine was the earliest approved vaccine in Switzerland that was made available primarily for individuals at high risk of severe COVID-19. By the time health-care professionals and the general population became eligible for SARS-CoV-2 vaccination, the mRNA-1273 vaccine constituted the largest share of vaccines delivered to Switzerland. Finally, the study population was highly heterogeneous with regard to underlying diseases, indications for CD20 depletion treatment, and immunosuppressive co-medication. However, this complex study population represents a real-world scenario and provided a unique opportunity to explore routine laboratory variables that are readily available and could be used to predict vaccination efficacy and therefore guide vaccination timing despite the complexity of the various immunosuppressive regimens.

Based on the current data, we propose that a simple peripheral count of $CD4^+$ cells could serve as a starting point to stratify patients according to anticipated vaccination response, even in patients with recent or severe B-cell depletion, or both. Given the broad availability of SARS-CoV-2 vaccines to date in many countries and their good tolerability, vaccination should be offered to all patients, including immunocompromised patients, if a successful immune response can be predicted with an acceptable degree of certainty.

To conclude, the present study shows that patients with a history of B-cell-depleting anti-CD20 therapies, including rituximab and ocrelizumab, have a severely impaired humoral and cellular response to SARS-CoV-2 mRNA-based vaccines. Our analyses provide, to our knowledge, the first estimates of ideal peripheral $CD19^+$ and $CD4^+$ cell counts and time since last dose of anti-CD20 therapy that would allow a positive humoral response to SARS-CoV-2 vaccines. Following validation in independent cohorts in a prospective setting, these results could provide guidance for coordinating both the administration of SARS-CoV-2 vaccines and B-cell-depleting agents in this population.

Contributors

MBM, BM, and DS conceived the study. MBM, FRS, MPH, CH, BM, and DS designed the study. MBM, DA, JA, BMo, LN, CM, AAS, LB, SRH, SMSJ, AC, RH, VUB, LYM, BM, and DS recruited participants. FSR, MPH, and JMI did and supervised the laboratory analyses. MBM and DS verified the underlying data and MBM, FRS, MPH, CH, DS, and BM did the analyses. MBM, BM, and DS wrote the manuscript with input from all authors. All authors approved the final version of the manuscript. MM and DS directly accessed and verified the data. The authors assume responsibility for the accuracy and completeness of the data and analyses, as well as for the fidelity of the trial and this report to the study protocol.

Declaration of interests

We declare no competing interests.

Data sharing

Data are available from the authors on request. Please contact the corresponding author for access.

Acknowledgments

This study was funded by Bern University Hospital. The funder had no role in study design, interpretation of results, drafting of the manuscript, or the decision to publish the results. We thank all volunteers and patients for their participation, and all involved nurses and study nurses for their contribution to data collection. We also thank the statistical staff of the Clinical Trials Unit, University of Bern, for advice on the statistical analysis plan. We also thank all involved technicians and lab personnel for technical assistance.

References

1 O'Driscoll M, Ribeiro Dos Santos G, Wang L, et al. Age-specific mortality and immunity patterns of SARS-CoV-2. *Nature* 2021; **590:** 140–45.

2 Portolés J, Marques M, López-Sánchez P, et al. Chronic kidney disease and acute kidney injury in the COVID-19 Spanish outbreak. *Nephrol Dial Transplant* 2020; **35:** 1353–61.

3 Liang W, Guan W, Chen R, et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. *Lancet Oncol* 2020; **21:** 335–37.

4 European Medicines Agency. COVID-19 vaccines: key facts. https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/vaccines-covid-19/covid-19-vaccines-key-facts (accessed March 3, 2021).

5 Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. *N Engl J Med* 2021; **384:** 1412–23.

6 Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med* 2020; **383:** 2603–15.

7 Baker D, Roberts CAK, Pryce G, et al. COVID-19 vaccine-readiness for anti-CD20-depleting therapy in autoimmune diseases. *Clin Exp Immunol* 2020; **202:** 149–61.

8 Storz U. Rituximab: how approval history is reflected by a corresponding patent filing strategy. *MAbs* 2014; **6:** 820–37.

9 Strangfeld A, Schäfer M, Gianfrancesco MA, et al. Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann Rheum Dis* 2021; **80:** 930–42.

10 Wang X, Du W, Zhang X, Li P. The influence of different disease states on rituximab pharmacokinetics. *Curr Drug Metab* 2020; **21:** 938–46.

11 Deepak P, Kim W, Paley MA, et al. Glucocorticoids and B cell depleting agents substantially impair immunogenicity of mRNA vaccines to SARS-CoV-2. *medRxiv* 2021; published online April 9. https://doi.org/10.1101/2021.04.05.21254656 (preprint).

12 Thakkar A, Gonzalez-Lugo JD, Goradia N, et al. Seroconversion rates following COVID-19 vaccination among patients with cancer. *Cancer Cell* 2021; **39:** 1081–90.

13 Furer V, Eviatar T, Zisman D, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. *Ann Rheum Dis* 2021; published online June 14. https://doi.org/10.1136/annrheumdis-2021-220647.

14 Bonelli MM, Mrak D, Perkmann T, Haslacher H, Aletaha D. SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response. *Ann Rheum Dis* 2021; published online May 6. https://doi.org/10.1136/annrheumdis-2021-220408.

15 Spiera R, Jinich S, Jannat-Khah D. Rituximab, but not other antirheumatic therapies, is associated with impaired serological response to SARS- CoV-2 vaccination in patients with rheumatic diseases. *Ann Rheum Dis* 2021; published online May 11. https://doi.org/10.1136/annrheumdis-2021-220604.

16 Braun-Moscovici Y, Kaplan M, Markovits D, et al. Humoral response to Pfizer mRNA vaccine against SARS CoV2, in patients with autoimmune inflammatory rheumatic diseases and the impact on the rheumatic disease activity. *medRxiv* 2021; published online April 6. https://doi.org/10.1101/2021.04.02.21254493 (preprint).

17 Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021; **371:** eabf4063.

18 Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell* 2020; **183:** 158–68.

19 Painter MM, Mathew D, Goel RR, et al. Rapid induction of antigen-specific $CD4^+$ T cells guides coordinated humoral and cellular immune responses to SARS-CoV-2 mRNA vaccination. *bioRxiv* 2021; published online April 22. https://doi.org/10.1101/2021.04.21.440862 (preprint).

20 Kalimuddin S, Tham CYL, Qui M, et al. Early T cell and binding antibody responses are associated with COVID-19 RNA vaccine efficacy onset. *Med* 2021; **2:** 682–88.

21 Thimme R, Oberhardt V, Luxenburger H, et al. Rapid and stable mobilization of fully functional spike-specific CD8+ T cells preceding a mature humoral response after SARS-CoV-2 mRNA vaccination. *In Review* 2021; published online May 11. https://doi.org/10.21203/rs.3.rs-505193/v1 (preprint).

22 Bange EM, Han NA, Wileyto P, et al. $CD8^+$ T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nat Med* 2021; **27:** 1280–89.

23 Brigger D, Horn MP, Pennington LF, et al. Accuracy of serological testing for SARS-CoV-2 antibodies: First results of a large mixed-method evaluation study. *Allergy* 2021; **76:** 853–65.

24 Lippi G, Salvagno GL, Pegoraro M, et al. Preliminary evaluation of Roche Cobas Elecsys Anti-SARS-CoV-2 chemiluminescence immunoassay. *Clin Chem Lab Med* 2020; **58:** e251–53.

25 Meyer B, Torriani G, Yerly S, et al. Validation of a commercially available SARS-CoV-2 serological immunoassay. *Clin Microbiol Infect* 2020; **26:** 1386–94.

26 Van Praet JT, Vandecasteele S, De Roo A, De Vriese AS, Reynders M. Humoral and cellular immunogenicity of the BNT162b2 mRNA Covid-19 vaccine in nursing home residents. *Clin Infect Dis* 2021; published online April 7. https://doi.org/10.1093/cid/ciab300.

27 Breheny P, Burchett W. visreg: visualization of regression models. Version 2.7.0. June 4, 2020. https://CRAN.R-project.org/package=visreg (accessed Dec 4, 2020).

28 Diefenbach C, Caro J, Koide A, et al. Impaired humoral immunity to SARS-CoV-2 vaccination in non-hodgkin lymphoma and CLL patients. *medRxiv* 2021; published online June 3. https://doi.org/10.1101/2021.06.02.21257804 (preprint).

29 Apostolidis SA, Kakara M, Painter MM, et al. Altered cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *medRxiv* 2021; published online June 30. https://doi.org/10.1101/2021.06.23.21259389 (preprint).

30 Simon D, Tascilar K, Schmidt K, et al. Brief report: humoral and cellular immune responses to SARS-CoV-2 infection and vaccination in B cell depleted autoimmune patients. *Arthritis Rheumatol* 2021; published online July 1. https://doi.org/10.1002/art.41914.

31 Maneikis K, Šablauskas K, Ringelevičiūtė U, et al. Immunogenicity of the BNT162b2 COVID-19 mRNA vaccine and early clinical outcomes in patients with haematological malignancies in Lithuania: a national prospective cohort study. *Lancet Haematol* 2021; **8:** e583–92.

32 Ramwadhdoebe TH, Boumans MJH, Bruijnen STG, et al. A8.10: the effect of rituximab treatment on B and T cell subsets in lymphoid tissues of patients with rheumatoid arthritis. *Ann Rheum Dis* 2015; **74** (suppl 1)**:** A85.

33 Hardeman P, Mann M, Hughes S, Greenberg B. Does rituximab cause depletion of T-cells in multiple sclerosis and neuromyelitis optica? (P2.158). *Neurology* 2016; published online April 4. https://n.neurology.org/content/86/16_Supplement/P2.158 (accessed Aug 3, 2021).

34 Levenson E, Howard J. What we know about Moderna's coronavirus vaccine and how it differs from Pfizer's. *CNN*, Dec 20, 2020. https://www.cnn.com/2020/12/17/health/moderna-vaccine-what-we-know/index.html (accessed July 3, 2021).