CLINICAL SCIENCE

Additional heterologous versus homologous booster vaccination in immunosuppressed patients without SARS-CoV-2 antibody seroconversion after primary mRNA vaccination: a randomised controlled trial

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ABSTRACT

Objectives SARS-CoV-2-induced COVID-19 has led to exponentially rising mortality, particularly in immunosuppressed patients, who inadequately respond to conventional COVID-19 vaccination.

Methods In this blinded randomised clinical trial, we compare the efficacy and safety of an additional booster vaccination with a vector versus mRNA vaccine in non-seroconverted patients. We assigned 60 patients under rituximab treatment, who did not seroconvert after their primary mRNA vaccination with either BNT162b2 (Pfizer–BioNTech) or mRNA-1273 (Moderna), to receive a third dose, either using the same mRNA or the vector vaccine ChAdOx1 nCoV-19 (Oxford–AstraZeneca). Patients were stratified according to the presence of peripheral B cells. The primary efficacy endpoint was the difference in the SARS-CoV-2 antibody seroconversion rate between vector (heterologous) and mRNA (homologous) vaccinated patients by week 4. Key secondary endpoints included the overall seroconversion and cellular immune response; safety was assessed at week 1 and week 4.

Results Seroconversion rates at week 4 were comparable between vector (6/27 patients, 22%) and mRNA (9/28, 32%) vaccines (p=0.6). Overall, 27% of patients seroconverted; specific T cell responses were observed in 20/20 (100%) vector versus 13/16 (81%) mRNA vaccinated patients. Newly induced humoral and/or cellular responses occurred in 9/11 (82%) patients. 3/37 (8%) of patients without and 12/18 (67%) of the patients with detectable peripheral B cells seroconverted. No serious adverse events, related to immunisation, were observed.

Conclusions This enhanced humoral and/or cellular immune response supports an additional booster vaccination in non-seroconverted patients irrespective of a heterologous or homologous vaccination regimen.

INTRODUCTION

The current pandemic caused by SARS-CoV-2 has led to exponentially rising morbidity and mortality worldwide. Apart from aggressive quarantine and infection control hygiene measures, the most effective way to combat SARS-CoV-2 spread is a population-wide vaccination strategy, foremost in those at high risk to develop severe COVID-19. Two types of vaccines have been currently approved by the European Medicines Agency: vector vaccines, such as ChAdOx1 nCoV-19 (Oxford–AstraZeneca) and Ad26.COV2-S (Johnson & Johnson), and mRNA vaccines, such as BNT162b2 (Pfizer–BioNTech) and mRNA-1273 (Moderna). However, immune responses to these vaccines vary between individuals, and antibody levels wane over time.

Key messages

What is already known about this subject?

► A third COVID-19 vaccination has been recommended by the US Food and Drug Administration for certain immunocompromised individuals.

► First clinical trial data have now reported on efficacy of a third vaccination in patients under immunosuppressive therapy.

► No clinical trial data exist which compare efficacy and safety of a heterologous versus homologous vaccination strategy in non-seroconverted patients under rituximab therapy.

What does this study add?

► The results from our study support efficacy and safety of an additional heterologous or homologous booster vaccination in immunosuppressed patients.

► Cellular and humoral immune response can be induced in B cell depleted patients undergoing rituximab treatment.

How might this impact on clinical practice or future developments?

► Based on these data, COVID-19 booster vaccination is recommended for non-seroconverted rituximab-treated patients.
Application of an additional booster dose has most recently been investigated. Several countries already started a third vaccination, especially in patients at high risk. Most recently, the US Food and Drug Administration authorised an additional vaccine dose for certain immunocompromised patients.

Patients under immunosuppressive therapy with rituximab, a B cell depleting monoclonal antibody against the CD20 surface antigen, are at a high risk for severe COVID-19 requiring hospitalisation and ICU admission. At the same time, B cell depletion reduces immune responses to vaccination. This combination poses a dilemma and, therefore, a highly unmet clinical need for this group of patients. Those lacking B lymphocytes in the periphery at the time of vaccination and thus did not yet start reconstituting their B cell pool often fail to seroconvert. Although B cell depleted patients can develop a T cell response, to date, it is unclear to what extent cellular and humoral responses contribute to protection against SARS-CoV-2 infection.

The development of a humoral immune response currently constitutes a good surrogate of protection and its absence is, therefore, often considered an alarm signal for an insufficient vaccination response. In order to stimulate the humoral immune response of rituximab-treated patients who do not respond to the conventional scheme of COVID-19 vaccination, an additional booster vaccination may be an obvious clinical strategy. Recent studies also evaluated the safety and efficacy of homologous versus heterologous schemes for primary and secondary vaccination in healthy individuals. However, it is unknown whether a heterologous approach could benefit those who completely lack a humoral immune response after basic immunisation. Furthermore, no data exist on responses to an additional booster vaccination in patients who had completely failed to mount a specific antibody response after the primary two-vaccination schedule.

In this blinded, randomised, controlled trial, we addressed this question and the general inducibility of a humoral or T cell response in rituximab-treated autoimmune disease patients without anti-SARS-CoV-2 antibodies after their basic mRNA vaccination.

METHODS

In this prospective patient and efficacy (laboratory), blinded randomised controlled trial adults (≥18 years) with chronic inflammatory rheumatic or neurologic diseases under current rituximab therapy and without detectable SARS-CoV-2 spike (S) protein antibodies at least 4 weeks after their second standard vaccination with an mRNA vaccine (BNT162b2 or mRNA-1273) were included. Key exclusion criteria were previous infection with SARS-CoV-2 or known allergies to study compounds. The detailed inclusion/exclusion criteria can be found in the trial protocol (online supplemental file 1). The trial was registered...
Figure 2  Antibody seroconversion rate 4 weeks after vector vs mRNA booster vaccination. Antibodies to the RBD of the viral S protein were determined using an anti-SARS-CoV-2 immunoassay. (A) Seroconversion rate was calculated based on the presence of anti-RBD antibodies in patients stratified by booster vaccination with vector vaccine or mRNA vaccine, in all patients and in patients with and without detectable peripheral B cells. (B) Anti-RBD antibody levels in patients with (n=18) and without (n=37) peripheral B cells, with colour of the circles indicating the type of vaccine. (C) Anti-RBD antibody levels in patients 4 weeks after booster vaccination with vector vaccine (n=27) or mRNA vaccine (n=28), with colour of the circles indicating the presence or absence of detectable peripheral CD19+ B cells. RBD, receptor-binding domain; S, spike.

with EudraCT (2021-002348-57) on 10 May before inclusion of the first patient.

Randomisation
Patients were block-randomised in a 1:1 ratio based on the presence or absence of peripheral B lymphocytes by a computerised randomisation algorithm to receive either a third dose of an mRNA vaccine (BNT162b2 or mRNA-1273, respectively) or a third vaccination with a vector COVID-19 vaccine (ChAdOx1 nCoV-19).

Interventions
During the screening visit (visit one), data on demographics, concomitant medication, possible hypersensitivity reactions to the previous SARS-CoV-2 vaccination and medical history regarding SARS-CoV-2 infections were collected. The absence of detectable SARS-CoV-2 antibodies against nucleocapsid and S protein was verified before enrolment and the level of peripheral B lymphocytes was assessed. The vaccination was applied during a baseline visit (visit two, within 28 days after screening) followed by visits 3 and 4 (1 week and 4 weeks after vaccination, respectively) to determine the efficacy and safety of the third COVID-19 vaccination. Serum samples obtained during visits 1, 3 and 4 were stored below −70°C at the Biobank of the Medical University of Vienna, a centralised facility for the preparation and storage of biomaterial with certified quality management (International Organization for Standardization (ISO) 9001:2015).27 Peripheral blood mononuclear cells were isolated at screening and week 1 by density gradient centrifugation and stored in the vapour phase of liquid nitrogen until further use.

All patients were blinded throughout visit 4, mainly to allow objectivity in safety assessment of the two strategies; blinding of vaccines was ensured by using pre-arranged dose aliquots in syringes without reference to the type used by the Central Pharmacy of the Vienna General Hospital. The City of Vienna provided the vaccines for this study free of charge. The study was conducted in following Good Clinical Practice guidelines and the Declaration of Helsinki. All trial visits were conducted in a tertiary hospital (Vienna General Hospital). The first patient was included on 25 May 2021 and the last patient finalised the 4 week follow-up on 5 August 2021.

Patient and public involvement
Patients and/or the public were not involved in the design, conduct, or reporting, or dissemination plans of this research.

Assessment, outcomes and sample size
The quantification of CD19+ peripheral B cells, the anti-SARS-CoV-2 antibody testing and T cell assays is detailed in the methods section of the online supplemental file 2. Laboratory assessors were blinded to randomisation.

The primary study endpoint was defined as difference in antibody seroconversion rates between the vector and mRNA vaccinated groups.

Secondary endpoints included seroconversion rate and SARS-CoV-2 antibody levels at week 4 overall and stratified for patients with and without detectable peripheral B cells as well as cellular immune response defined by T lymphocyte restimulation potential before and 1 week after vaccination. Safety was reported and evaluated for incidence and severity of adverse events as well as potential effects on the underlying disease activity over a period of 28 days. Additionally, a paper-based patient diary was used. The study sample size was pragmatically targeted at 60 individuals, based on the number of rituximab-treated patients potentially eligible during the tight recruitment period, including estimates of non-responders to a standard protocol of mRNA vaccination, and expected participation rates. Based on a χ2 test comparing vector versus mRNA vaccine, this number of patients would allow to achieve at least 80% power at a minimal detectable difference of 28% (5% of responders in one group vs 33% in the other).

Statistical analysis
All subjects vaccinated with a third dose were included in the analysis. Primary outcome was assessed using χ2 test. Secondary outcomes and safety data are presented in a descriptive manner. Post-hoc exploratory analyses were performed to evaluate factors associated with seroconversion rates by univariate logistic regression analyses. Variable selection was based on previous data in rituximab-treated patients, and included age, concomitant medication, type of booster vaccination and the presence or absence of detectable peripheral B cells.22 GraphPad Prism (V9.1.0) was used for the graphical presentation of the data. R V4.0.3 (R Development Core Team, Vienna, Austria) was used for the entire statistical analysis. Following
Sixty-eight patients under rituximab treatment who had been immunised with two doses of mRNA vaccine were screened for eligibility. Eight patients were excluded due to the presence of detectable SARS-CoV-2-specific antibodies. Sixty non-seroconverted patients were randomised, of whom 30 were assigned to receive vector vaccine and 30 to receive mRNA vaccine as the third dose; 5 patients withdrew consent between screening and baseline visit (figure 1). A total of 27/30 patients were vaccinated with a vector vaccine and 28/30 received an mRNA vaccine. All patients subsequently presented at follow-up visits and completed the trial at week 4 after vaccination. Patient characteristics were similar between the two randomised groups (table 1).

Seroconversion rates at week 4 were numerically lower in the vector group than in the mRNA group (6/27, 22% of patients compared with 9/28, 32% of patients) (figure 2A). Despite the numerical difference in favour of the homologous vaccination group, disadvantage of the heterologous group cannot be supported statistically (p=0.6).

Even though the primary endpoint was not met, 27% of all vaccinated patients seroconverted independent of the vaccine used with a median SARS-CoV-2 S antibody level of 15.7 BAU/mL (IQR: 4.7, 25.8 BAU/mL). Neutralising antibodies (titre ≥10) against SARS-CoV-2 were observed in 4/15 (27%) of all seroconverted patients. Seroconversion rate was higher in patients with detectable peripheral CD19+ B cells versus those without (figure 2A). Among patients with no detectable peripheral B cells (37/55, 67%), antibodies to the receptor-binding domain (RBD) of the viral S protein (anti-RBD antibodies) were detectable in 3/37 (8%) patients; in patients with detectable peripheral B cells, seroconversion rate was 67% (12/18) at week 4 (figure 2A–C). Median levels of anti-RBD antibodies were 19.4 (IQR: 8.2, 114.8) and 12.4 (IQR: 3.8, 17.8), respectively, in seroconverted vector and mRNA vaccinated patients (figure 2B; online supplemental table 1).

SARS-CoV-2-specific T cell responses were determined by enzyme-linked immune absorbent spot (ELISpot) assay in all
patients before and after booster vaccination. Matched samples before and after the third vaccination were available from 36 patients. Patient characteristics for this group stratified by third vaccination are presented as online supplemental table 2. At screening, 15/20 (75%) of patients assigned to the vector group and 10/16 (63%) assigned to the mRNA group had detectable S-specific T cells. Administration of a third vaccine dose led to an increase to 20/20 (100%) in the vector group and 13/16 (81%) in the mRNA group (figure 3A–B). The number of spot-forming cells to the S peptide pools (S1/S2) was slightly higher after boosting with vector vaccine (median: 459, IQR: 133, 722) as compared with mRNA vaccine (median: 305, IQR: 171, 416) (figure 3C).

Integrative analysis of humoral and T cell responses for 36 patients with matched samples before and after the third vaccination was performed: before third vaccination, 11/36 patients (31%) had neither anti-RBD antibodies nor T cell response (AB−, T−), and 25/36 patients (69%) did not have a humoral but exhibited a cellular immune response (AB−, T+). After the third vaccination, 8/36 (22%) showed a humoral and T cell response (AB+, T+), 1/36 (3%) had a humoral but no detectable cellular immune response (AB+, T−), in 25/36 (69%) a cellular but no humoral immune response (AB−, T+) was observed and 2/36 (6%) developed neither a humoral nor a cellular immune response. Overall, a cellular and/or humoral immune response could be achieved through an additional booster vaccination strategy with two doses of mRNA vaccine (figure 3D, online supplemental tables 2 and 3).

Exploratory post-hoc univariate logistic regression models revealed that detectable peripheral B cells strongly favoured the likelihood of seroconversion (OR: 22.67, 95%CI 5.46 to 125.10), while co-medication with any conventional synthetic disease-modifying antirheumatic drug (csDMARD) favoured non-seroconversion. Compared with mRNA booster vaccination, the vector vaccine showed a lower likelihood of inducing humoral response though not statistically significant. With respect to T cell response, no association with age, use of prednisone or the presence of peripheral B cells could be observed (figure 4). All patients vaccinated with the vector regimen developed a T cell response, while all patients without T cell response were co-treated with a csDMARD, resulting in non-convergence of the respective regression models (online supplemental table 4).

Systemic reactogenicity was evaluated by the patients using a paper-based diary daily during the first week after vaccination. Adverse events, in general, were monitored until 28 days after vaccination. One serious adverse event was reported after the screening visit prior to vaccination. Most side effects were similar between vector and mRNA booster vaccine groups. Numerically, a higher prevalence of systemic reactogenicity after the booster dose was reported by patients in the heterologous vaccine group compared with homologous vaccine schemes for fatigue, arthralgia and myalgias. Thirteen out of 27 (48%) of vector-vaccinated patients developed arthralgias as compared with 8/28 (29%) of patients with mRNA booster vaccination. Myalgia was reported in 15/27 (56%) of vector-vaccinated patients compared with 9/28 (32%) of mRNA-vaccinated patients. Fatigue was
present in 21/27 (78%) of vector-vaccinated patients, while only 13/28 (46%) of mRNA-boosted patients experienced fatigue. Local pain at the injection site was more frequent during the first 2 days in mRNA-vaccinated patients (16/28, 57%) than vector-vaccinated patients (8/27, 30%). The local and systemic reactogenicity for the first week after vaccination is displayed in figure 5.

No thrombocytopenia or antibodies against platelet factor 4 were observed after additional booster vaccination 1 week and 4 weeks after vaccination. Mean thrombocyte counts were 283 G/L ± 85 before and 296 G/L ± 79 one week after vaccination. None of the patients experienced any anaphylactoid reaction or neurological complication. Seven patients (13%) reported an alteration or worsening in their underlying disease 1 week after vaccination, but no disease flare that required glucocorticoid treatment or change in immunosuppressive medication was reported within the study period.

**DISCUSSION**

In this randomised, controlled clinical trial, we enrolled patients treated with rituximab for an underlying autoimmune disease, who had not seroconverted on vaccination with two doses of an mRNA vaccine, and thus continued to be at a high risk for a severe disease course of SARS-CoV-2 infection. The additional SARS-CoV-2 booster vaccination evaluated in this trial resulted in the development of a humoral immune response in 27% of this initially vaccination-refractory patient population. Moreover, the additional booster vaccination reduced the proportion of patients lacking both a humoral and cellular immune response to primary vaccination from 31% to 6%.

Currently approved vector and mRNA vaccination strategies against SARS-CoV-2 consider only homologous vaccination. However, recent studies indicate a better humoral and cellular immune response after heterologous prime-boost vaccination in healthy individuals. Reactogenicity was similar on third vaccination as reported previously. In our trial, typical general systemic reactions (like fever, myalgia and similar) were observed that were within the scope of the reports from the large approval studies with some numerical differences seen between the two treatment groups.

One limitation of the trial is the absence of a placebo control, which was considered unethical in this high-risk population. While the small sample size precluded delivering ultimate statistical evidence concerning the clinical question of differences in immune responses to booster vaccination with homologous vs heterologous products, the important result of our study is that a third booster vaccination is effective in inducing an immune response in these refractory patients. Since we cannot generalise these data to the wider population of non-responders to COVID-19 vaccines, that is, beyond immunosuppressed patients, broader population-based programmes are needed to evaluate the impact of an additional booster vaccination in non-responding healthy individuals. It is important to note, that it still needs to be determined how humoral and cellular immune responses (or their absence) relate to protection against clinical infection with SARS-CoV-2.

Our data show that a cellular and/or humoral immune response can be achieved on a third COVID-19 vaccination in most of the patients who initially developed neither a humoral nor a cellular immune response. The efficacy data together with the safety data seen in our trial provide a favourable risk/benefit ratio and support the implementation of a third vaccination for non-seroconverted high-risk autoimmune disease patients treated with B cell depleting agents. This might be a viable way to protect this group of patients from more dire consequences of an acquired SARS-CoV-2 infection.

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