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Humoral and cellular immune responses to standard and third dose SARS-CoV-2 vaccination in rituximab treated rheumatoid arthritis patients – a prospective cohort study

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SUMMARY

Background

Humoral- and cellular immune responses following standard two dose SARS-CoV-2 vaccination in rheumatoid arthritis (RA) patients treated with rituximab are not well characterised, and data on third dose vaccination in this patient group are currently lacking.

Methods

This prospective observational study included RA patients (n=87) on rituximab therapy and healthy controls (n=1114) receiving standard two dose SARS-CoV-2 vaccination. Patients with a weak serological response were allotted a third vaccine dose (n=49). Serum samples collected prior to, and after vaccination were analysed for antibodies to the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein. Vaccine-elicited T cell responses were assessed in vitro by challenging cryo-preserved Peripheral Blood Mononuclear Cells (PBMCs) with the Spike protein in a subset of patients (n=19).

Findings

19 (22%) patients compared to 1096 (98%) healthy controls ($p<0{\cdot}0001$) had a serological response after standard SARS-CoV-2 two dose vaccination. The main determinants for a humoral response in patients was time since last rituximab infusion (median 267 days [IQR 222–324]) and vaccine type mRNA-1273 as compared to BNT162b2. Following standard vaccination 10/19 (53%) and 14/19 (73%) of patients presented $CD4^+$ and $CD8^+$ T cell responses, respectively. A third vaccine dose induced serological response in only 8/49 (16%) patients, but $CD4^+$ and $CD8^+$ T cell responses in all patients assessed (n=12), including responses to the SARS-CoV-2 delta variant (B.1.617.2). Adverse events were reported in 48% patients and 78% healthy controls after standard vaccination with no increased frequency after the third dose.

Interpretation

This study provides important insight into the diverging humoral and cellular responses to standard- and third dose SARS-CoV-2 vaccination in rituximab treated RA patients. A third vaccine dose given within 6–9 months after a rituximab infusion will likely not induce a serological response, but could be considered in order to boost the cellular immune response.

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INTRODUCTION

SARS-CoV-2 vaccines have proven efficient and safe in the general population,[1,2] but a good vaccine response depends on a functional immune system that includes concerted B cell and T cell responses. Immunosuppressive medications, and particularly rituximab, a $CD20^+$ cell depleting therapy, are known to impair immunogenicity of influenza and pneumococcal vaccines.[3] Rheumatoid arthritis (RA) patients on rituximab therapy are at a heightened risk of severe COVID-19 outcomes,[4-7] and it is of vital importance to evaluate their response to SARS-CoV-2 vaccination. Observational data in small RA cohorts have indicated that rituximab impairs serological SARS-CoV-2 vaccine responses.[8-11] Previous reports have suggested that T cells are necessary for protection against severe COVID-19 in settings of low antibody levels,[12] for rapid and efficient resolution of COVID-19,[13] and for protection against fatal outcomes in patients treated with anti-CD20 for haematological malignancies.[14] To date, limited data exist regarding cellular responses to SARS-CoV-2 vaccines in rituximab treated RA patients.[11,15] In the absence of a normal serological response, cellular immunity is of crucial interest in this patient group.

The utility of a third vaccine dose in immunocompromised patients, as well as in the general population, is an urgent question in the global medical community and for policy makers.[16,17] It is unclear if B cell depleted patients who lack serological response after standard two dose vaccination will benefit from a third vaccine dose. While a recent case series on rituximab treated patients indicated limited benefit from a third dose,[18] no data exist on cellular response or safety following a third vaccine dose in these patients.

The aim of the present study was to assess the humoral- and cellular responses and adverse events following standard and third-dose SARS-CoV-2 vaccination in RA patients treated with rituximab.

METHODS

Participants and study design

Nor-vaC (Norwegian study of vaccine response to COVID-19 vaccines in patients using immunosuppressive medication within rheumatology and gastroenterology) is an ongoing longitudinal observational study conducted at two Norwegian hospitals with large specialist clinics; the Division of Rheumatology and Research at Diakonhjemmet Hospital (DH) and the Department of Gastroenterology at Akershus University Hospital (AHUS). Eligibility criteria are presented in the appendix (p2). Eligible patients identified by hospital records received an invitation to participate in the study prior to initiation of the national vaccination programme in February 2021. In the present analyses we included RA patients on rituximab therapy. Healthy controls were blood donors and health care workers from collaborating hospitals in Oslo. The study was approved by an independent ethics committee (Regional Committees for Medical and Health Research Ethics South East, reference numbers 235424, 135924, 204104) and by appropriate institutional review boards. The study is registered at clinicaltrials.gov NCT04798625. All patients and healthy controls provided written informed consent.

During the conduct of this trial, patients included in the Nor-vaC study with anti-RBD levels <100AU/ml after standard two dose vaccination were recruited into a separate intervention study (EudraCT Number: 2021-003618-37) and allotted a third vaccine dose in July–August 2021. The present observational study reports humoral and cellular immune responses following the third dose in 49 RA patients treated with rituximab.

Study procedures

All participants received SARS-CoV-2 vaccines according to the Norwegian national vaccination programme. Three SARS-CoV-2 vaccines were available: BNT162b2, mRNA-1273 and ChAdOx1. The two mRNA vaccines were given with an interval of 3–6 weeks

between the 2 doses. The ChAdOx1 vaccine was withdrawn from the Norwegian vaccination programme in March 2021 and all persons who had received one dose of this vaccine received one of the mRNA vaccines as the second dose. The vaccines were administered to the participants following a priority list given by the Norwegian Institute of Public Health (NIPH). According to the programme, persons recovered from COVID-19 infection received one vaccine dose only.

Patients receiving a third dose were asked to pause their concomitant DMARD treatment one week before and two weeks after vaccination.

Data collection

Informed consents and questionnaires were collected through "Services for Sensitive Data" (TSD) at the University of Oslo (UiO). Participating patients were asked to complete questionnaires before vaccination and approximately 14 days after the first, second and third vaccine dose. Demographic data including diagnosis, age, sex, weight, height and smoking status were collected at baseline. Information regarding medication use, patient reported disease activity and COVID-19 related questions (symptoms, test results and hospitalisation), pausing of medication at the time of vaccination, as well as adverse events after vaccinations, were collected at baseline and 14 days after the first, second and third vaccine dose. Date of the last rituximab infusion, total number of rituximab infusions, disease- and rituximab duration, co-medication and number of previous DMARDs were obtained from the medical records. Disease activity (disease activity score (DAS 28), patient global assessment (PGA), and physician global assessment (PhGA)) were assessed 2–4 weeks after the second vaccine dose. For most healthy controls, only information on vaccine date and type, participant sex and age were collected. 246 controls (health care workers at DH and AHUS) additionally answered detailed questionnaires on demographic data and adverse events.

153 Information about vaccination dates and type of vaccines were obtained from the Norwegian
154 Immunisation Registry, SYSVAK.[19] Information regarding patients testing positive for
155 COVID-19 disease prior to and during the study period was obtained from the Norwegian
156 Surveillance System for Communicable Diseases (MSIS).[20]

157 **Serological analyses**

158 Antibodies to the full-length Spike protein from SARS-CoV-2 and the receptor-binding
159 domain (RBD) were measured 2–4 weeks after standard two dose vaccination and after third-
160 dose vaccination using an in-house bead-based method (see appendix pp3–4 for a detailed
161 description).[21] We defined antibody levels above the two-percentile of standard vaccinated
162 healthy individuals, corresponding to levels ≥ 70 AU/ml as response.[22] Levels < 5 AU/ml
163 were defined as no response, while levels between 5–70 AU/ml were defined as weak
164 response. Calibration to the WHO international standard showed that 70 AU/ml corresponds
165 to approximately 40 Binding Antibody Units per millilitre (BAU/ml).

166 **Analyses of T cell responses**

167 Prior to the first vaccine dose, a subset of randomly chosen patients (n=20) were asked to
168 provide blood samples for cellular analysis before and 7–10 days after the second vaccine
169 dose. The number was based on the feasibility of performing complex cellular analyses and
170 the previous experience of the researchers performing them. 12 of the patients were recipients
171 of a third dose and additionally donated blood for cellular analyses 3 weeks after the third
172 dose. Thawed Peripheral Blood Mononuclear Cells (PBMCs) were stimulated with SARS-
173 CoV-2 PepTivator Spike protein peptides (Miltenyi Biotec), Wuhan - wild type, or the delta
174 variant (B.1.617.2), consisting of 15-mer sequences with 11 amino acids, overlap covering the
175 immunodominant parts of the spike protein, in the presence of costimulatory antibodies
176 against CD28 and CD49d (BD Biosciences) and Brefeldin-A (10 µg/mL, MilliporeSigma).

SARS-CoV-2-specific T cells were identified by dual expression of Tumour Necrosis Factor (TNF) and CD40L (CD154) for $CD4^+$ T cells and by single and/or dual intra-cellular expression of Interferon Gamma (IFNγ) and TNF for $CD8^+$ T cells. All samples were acquired on an Attune NxT (Thermofischer) flow cytometer and analysed using FlowJo version 10 software. See appendix (pp5–6) for a detailed description of the methodology.

Objectives and endpoints

The two main objectives of this study were: a) to assess humoral and T cell responses to standard and third dose SARS-CoV-2 vaccination in RA patients on rituximab therapy as compared to healthy controls, and b) to assess changes in humoral and T cell responses after a third vaccine dose given to patients on rituximab therapy with weak serological responses to standard vaccination. Other objectives were to assess safety of standard and third dose vaccination and to identify predictors of serological response in patients. The main endpoints were; a) the proportion of participants with serological response (anti-RBD levels >70AU/mL) and T cell responses to Spike peptides following standard and third dose SARS-CoV-2 vaccination; and b) the change in levels of anti-RBD and T cell responses to Spike peptides after third dose vaccination. Other endpoints included adverse events and predictors of serological response to standard and third dose vaccination.

Statistical analyses

Demographic data, adverse events and serological response were summarised using descriptive statistics. Comparisons of serological response between patients and controls were performed by logistic regression. Adjustments were made for sex, age and vaccination type. Comparison between pre- and post-vaccination samples in patients receiving a third dose was performed by Wilcoxon paired sampled test. GraphPad Prism Paired analysis and the Wilcoxon matched-pairs signed rank test was used to compare the frequencies of antigen-

specific T cells. Comparisons of potential risk factors between response groups (table 3) were performed by Kruskal-Wallis/Fishers exact test for continuous/categorical outcomes. To assess predictors of serological response to vaccine in patients, uni- and multivariable logistic regression analyses were performed. Relevant variables were chosen by the investigators after a review of the existing literature. For multivariable model building, all factors with a p-value of less than 0·15 from univariable analyses as well as age and sex were included. The final model was obtained with significant variables only by backward elimination of the least significant variable. Spearman correlation test was used to compare T cell responses versus age and time since last rituximab infusion in patients. All tests were two-sided and conducted at the 0·05 significance level. Analyses were carried out using Stata v16, GraphPad Prism version 9 and R 3.4.4.

Role of the funding source

Nor-vaC was an investigator-initiated study with no initial funding. Subsequent funders of the study had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit the paper for publication.

RESULTS

A total of 90 RA patients treated with rituximab were enrolled between February 9, 2021, and May 27, 2021. 87 patients (median age 60 years [IQR 55–67]; 69 women [79%]) donated serum obtained at a median of 16 [IQR 12–21] days after the second vaccine dose, and were included in the present analyses. In addition, control samples from 1114 healthy health care providers and blood donors (median age 43 [IQR 32–55]; 854 women [77%]) were included. Baseline characteristics are shown in table 1. 56 (64%) of all patients used a conventional

systemic disease modifying drug (csDMARD) concomitantly, including; methotrexate (n=42), leflunomide (n=9), sulfasalazine (n=4), and hydroxychloroquine (n=1). 14 (17%) of all patients used prednisolone as co-medication, all with a dosage <10 mg/day. Patients were vaccinated with BNT162b2 (n=63, 72%), or mRNA1273 (n=21, 24%). Three patients had undergone COVID-19 prior to vaccination and received only one vaccine dose. No patients developed COVID-19 disease after standard two dose or third dose vaccination.

19 (22%) patients as compared to 1096 (98%) of healthy controls had anti RBD-levels ≥70 AU/ml defined as response after standard two dose vaccination (p<0·0001). 14 (16%) patients and 14 (1%) controls had a weak response, and 54 (62%) patients and 4 (0·4%) of healthy controls had no detectable antibodies (table 2, figure 1A). The median time between last rituximab infusion and first vaccine dose was 267 days (IQR 222–324) for patients with response, whereas the median intervals for patients with weak- and no response were 137 days (61–233) and 107 days (80–152), respectively (table 3, figure 1B). Univariable logistic regression identified the following variables as significantly associated to humoral response (appendix p8): The interval between last rituximab infusion and first vaccine dose by 100 days (OR 2·37 [95% CI 1·45–3.89], p=0·0005), CD19 cell count (OR 1·02 [95% CI 1·00–1.03], p=0·026), and vaccine type mRNA-1273 as compared to BNT162b2 (OR 3·81 [1·26–11·52], p=0·016). In the multivariable logistic regression model (appendix p8), the interval between last rituximab infusion and first vaccine dose by 100 days (OR 2·97 [95% CI 1·67–5·29], p=0·0002), and vaccine type mRNA-1273 compared to BNT162b2 (OR 9·12 [2·15–38·62], p=0·0022) were significant predictors of mounting a serological response when adjusted for age and sex (appendix p8).

49 patients (median age 62 years [IQR 56–67], 43 females [88 %]) with no or weak serological responses to standard two dose vaccination were allotted a third vaccine dose a median of 70 days [IQR 49–104] after the second vaccine dose. In these patients, median

antibody levels were 2 AU/ml [IQR 2–3] and 3 AU/ml [2–18] after the second and third vaccine dose, respectively (figure 1C). Comparison between antibody levels in pre- and post-vaccination samples showed a median change of 0·96 AU/ml (IQR 0·05–27), ($p<0·0001$). Eight (16%) patients, with a median interval between last rituximab and third dose of 250 days [IQR 206–265], achieved anti-RBD≥70 AU/ml defined as response after the third dose (table 2, figure 1C and 1D, appendix p7). Two patients had received only one vaccine dose due to prior COVID-19 infection and hence received their second dose, none of these developed a response. No significant predictors of serologic response after the third dose were found, possibly due to the low numbers of patients with response (n=8).

T cell responses were analysed in 19/20 patients after the second vaccine dose. 12/19 patients were further assessed after a third dose. After standard vaccination, 10/19 (53%) and 14/19 (73%) of patients presented $CD4^+$ and $CD8^+$ T cell responses, respectively (figure 2A). Patients without anti-Spike $CD8^+$ T cell response (n=5, 27%), also lacked detectable anti-Spike $CD4^+$ T cells. Time since last rituximab infusion was not associated with T cell response (data not shown). The reduced T cell responsiveness to the vaccine could not directly be explained by the regimen of immune-suppressive drugs (rituximab mono-therapy or combined with csDMARD) because the activation induced by a polyclonal stimulation of the TCR (with Cytostim) was similar between all donors, indicating normal functional responses (data not shown). T cell responses were detected in all vaccinated healthy donors (n=20) after their second dose of the vaccine, response magnitudes were similar to that seen in the patients (figure 2A).

After the third dose, all 12 patients had detectable anti-Spike $CD4^+$ and $CD8^+$ T cell responses, this included 5 of the patients who lacked T cell responses after the second dose (figure 2A).

In order to evaluate the potential of vaccines to induce a cross-protection against currently circulating viral strains, we extended the T cell analysis challenging PBMCs from vaccinated patients with Spike peptides derived from SARS-CoV-2 delta variant (B.1.617.2). The magnitude of T cell responses to B.1.617.2 Spike correlated with the levels of response towards wild type Spike for both $CD4^+$ and $CD8^+$ responses after second and third dose (figure 2B, Spearman analysis: $CD4^+$ dose 2: r=0·84, p<0·0001; $CD4^+$ dose 3, r=0·63, p<0·029; $CD8^+$ dose 2: r=0·73, p<0·0001; $CD8^+$ dose 3, r=0·71, p<0·012). Next, we compared helper and cytotoxic cellular response in RA patients. Combined anti-Spike T cell responses directed against wild type and delta SARS-CoV-2 Spike variant peptides are shown in figure 2C. The positive correlation between $CD4^+$ T cell responses and $CD8^+$ T cell responses, suggested that the vaccine elicited concerted T cell immunity (Spearman, r=0·64, p<0·0001). The age of the patients negatively correlated with the frequency of anti-Spike $CD4^+$ T cells (figure 2C, Spearman r=-0·33, p=0·0080).

After standard vaccination, adverse events (AE) were reported in 32 (48%) of patients and 191 (78%) of healthy controls, and in 19 (42%) of patients receiving a third dose (figure 3 and appendix p9). In patients who received a third vaccine dose, the number of adverse events was similar after second and third doses, with the exception of haematological AEs, where bleeding/bruises was more frequently reported after the third dose (4 patients (2%) to 7 patients (16%)). Among patients who received a third dose, 5 (14%), 3 (8%) and 7 (16%) patients reported disease flare after the first, second and third dose, respectively (appendix p9). There were no deaths among patients during the study period.

DISCUSSION

This large observational study is the first to report immunogenicity and safety following both standard two dose and third dose SARS-CoV-2 vaccination in rituximab treated RA patients. After standard vaccination only 22% of patients compared to 98% of healthy controls developed a humoral response. We found that despite these severely attenuated humoral responses and the absence of $CD19^+$ B cells, T cell responses were present in 73% of rituximab-treated patients after standard two dose vaccination and in all patients after the third dose. Patient responses to wild type Spike correlated with that seen towards the SARS-CoV-2 delta variant (B.1.617.2) S peptides, demonstrating that the vaccine had also elicited immunity to this variant. Both the standard two dose regimen and a third dose were safe in terms of patient-reported adverse events. To date, this is the largest study to combine sensitive measurements of humoral and cellular immunity as well as a description of adverse events after standard SARS-CoV-2 vaccination in RA patients treated with rituximab.

Previous studies have demonstrated a good correlation between the levels of neutralizing antibodies and protection from symptomatic COVID-19 disease.[23,24] However, serological responses decay with time after vaccination.[25] In contrast, SARS CoV-1 T cell memory is long-lasting and was found after 17 years.[26] A recent study in rhesus macaques demonstrated that T cell immune responses contributed to protection when antibody responses were low,[12] bridging insufficient humoral immunity. $CD4^+$ and $CD8^+$ T cells counteract viral infections by producing effector cytokines, such as IFNγ and TNF, and by exerting cytotoxic activity against virus-infected cells. Early and robust SARS-CoV-2-specific T cell responses were associated with lower severity of COVID-19.[13] Robust $CD8^+$ T cell responses were also associated with improved survival of COVID-19 in patients with hematologic malignancies, including patients on anti-CD20 therapies,[14] underlining the importance of T cell immunity in patients with impaired B cells.

We found that 53 % and 73% had $CD4^+$ and $CD8^+$ T cell responses after standard two dose vaccination. This is in line with a recent study of rituximab treated patients with various rheumatic diseases (IgG4 related disease, connective tissue diseases, vasculitis and rheumatoid arthritis) where 73% of patients had detectable IFNγ secreting SARS-CoV-2-specific T cells and half of patients lacked a serological response.[9] That study, however, did not discriminate between $CD4^+$ and $CD8^+$ T cells. In the current study we found a greater deficit in $CD4^+$ T helper cell responses that are required for optimal B cell responses after the second dose of the vaccine.

In patients with insufficient serological responses to the standard two dose vaccine regimen, we found that only a few patients mounted a serological response after a third dose. In contrast, the third dose induced both anti-Spike $CD4^+$ and $CD8^+$ T cells in all patients tested, regardless of humoral responses. The coordinated development of helper and cytotoxic T cell response might constitute protective immunity against future infections by SARS-CoV-2 and its variants. Our results suggest that the third dose enables robust T cell immunity in RA patients treated with rituximab, potentially improving protection in this patient group.

The present data show that time since last rituximab infusion was the most important factor predicting serological responses to standard SARS-CoV-2 vaccination, patients with response having a median interval of almost 9 months between last rituximab infusion and the first vaccine dose. This confirms findings in a recent study by Furer et.al. and observational data from smaller cohorts, demonstrating that the seroconversion rate in patients treated with rituximab increased from 20 to 50% when the interval between rituximab and SARS-CoV-2 vaccination increased from 6 to 12 months.[10,11] $CD19^+$ cell count was also associated with serological response to standard vaccination. This indicates that $CD19^+$ cell counts may be used as a surrogate for B cell function when timing vaccinations.

Vaccination with mRNA-1273 as compared to BNT162b2 was a significant predictor of response to standard vaccination (OR 9·12). This is in line with previous finding of higher humoral immunogenicity to mRNA-1273 compared to BNT162b2 in healthy subjects.[27]

Both standard and third dose vaccination were safe with respect to patient reported AEs, with no serious AEs reported. Numerically, patients on rituximab reported less adverse events than healthy controls. This might be due to lower age of the healthy controls, though we cannot rule out an association between AEs and humoral response, where immunosuppressive medication reduces side-effects as well as immunogenicity of the SARS-CoV-2 vaccines. More patients reported bleeding/bruises after the third than second dose, but sample size is small and the current results on adverse events should be interpreted with caution.

Strengths of this study include the broad inclusion with a personal invitation to all rituximab patients in our department which increases the generalisability of our findings; close follow-up including assessment of AEs, and broad assessment of vaccine response with both humoral - and in-depth cellular response to standard- and third dose vaccination.

This study also has some limitations. First, the study population was older (median 60 years) than the healthy controls (median 43 years), which might interfere with comparability of results. The difference in serological response, however, was greater than what can be explained by age alone,[28,29] and we adjusted for age in the analyses. Second, the number of included patients was too low to draw definite conclusions regarding safety, but our data are reassuring regarding the safety of third dose vaccination in immunocompromised patients with weak responses to standard vaccination. Third, for feasibility reasons, the number of patients with T cell assessments after the third dose included only 12 patients. However, patients chosen for T cell analyses were randomly selected among the rituximab patients prior to standard vaccination and our findings were consistent across all patients tested. Fourth,

only patients were offered a third dose, hence the response after a third dose could not be compared to healthy controls.

Rituximab treated RA patients are at risk of severe COVID-19 disease,[4,7] and are in particular need of protection by vaccination. In terms of serological responses, our data suggest that a prolonged interval between rituximab infusion and vaccination (>9 months) could be beneficial. Most rituximab-treated patients did not evolve a serological response to standard- or third dose vaccination, but exhibited a clear increase in the T cell response, and few adverse events upon receiving a third dose. Further studies are needed to assess the clinical protection provided by a cellular response in the absence of anti-SARS-CoV-2 antibodies, but our results raise the possibility that patients on regular rituximab infusions may rely on cellular immunity alone. This study supports third dose vaccination in rituximab treated RA patients in order to keep this vulnerable population protected against COVID-19, and can inform patients, health care providers and decision makers regarding the optimal vaccination strategy in rituximab treated RA patients.

RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed September 29, 2021, for studies published in English since January 1, 2020, using different combinations of the terms “Rheumatoid arthritis” “vaccination” “SARS-CoV-2” “COVID-19” “rituximab” and “response”. Prior observational studies are generally small, but indicate that rituximab impairs serological response to vaccines including SARS-CoV-2 vaccines, in patients with rheumatoid arthritis. Limited information exists on T cell responses, and no data exist on third dose vaccination on RA patients treated with rituximab.

Added value of this study

In this large rituximab RA cohort, only 22% of patients developed a normal serological response to standard two dose SARS-CoV-2 vaccination. Time between the last rituximab infusion and the first vaccine dose was the main predictor of a vaccine response with a median interval of nine months in patients with response. A cellular immune response was, however, present in more than half of the patients after standard two dose vaccination. A third vaccine dose given to patients with a weak serological response was safe and elicited a robust T-cell response in all patients, despite inducing a serological response in only a small proportion of patients.

Implications of all the available evidence

If possible, patients should be vaccinated against COVID-19 prior to initiation of rituximab therapy. For optimal response, the interval between rituximab and vaccination should be as long as possible, preferably at least 9 months. A cellular immune response after vaccination may be present in the absence of anti-SARS-CoV-2 antibodies.

A third vaccine dose given to a patient treated with rituximab within the last 6–9 months will likely not induce a serological response but could be considered in order to boost the cellular immune response. The clinical significance of the cellular immune response in the absence of

409 virus-specific antibodies remains to be elucidated. Alternative anti-rheumatic therapies might
410 be considered in individual patients if repeated rituximab infusions preclude the development
411 of protective anti-SARS-CoV-2 antibodies.
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CONTRIBUTORS

All authors critically revised the report and approved the final submitted version, and take the responsibility for the completeness and accuracy of the data and analyses. All authors had full access to all the data in the study and have made the final decision to submit the manuscript for publication. IJ, HK, GLG, SWS, ATT, FTJ, LAM and JS have verified the underlying data.

IJ, GLG, SWS, KKJ, FLJ, LAM and JTV conceived and designed the study. GLG, SWS, KKJ, FLJ, LAM, ATT, SAP and IJ oversaw the implementation of the study. GLG, SWS, SAP, KKJ, ATT and IJ collect the data. IJ, HK, GLG, SWS, FLJ, LAM, JS, ATT, SAP and IJ interpreted data and drafted the report. FLJ developed the assay used for serological assessment. FLJ, EBV and TTT performed the serological analysis. HK, SM and LAM performed the T-cell analysis. JS was the study statistician. ATT, DJW, TKK, EAH, SM, GG, GBK, and JJ contributed to study conception and design. LSHN-M and AMA contributed to data collection.

DATA SHARING

A de-identified patient data set can be made available to researchers upon reasonable request. The data will only be made available after submission of a project plan outlining the reason for the request and any proposed analyses, and will have to be approved by the Nor-vaC steering group. Project proposals can be submitted to the corresponding author. Data sharing will have to follow appropriate regulations.

DECLARATION OF INTERESTS

KKJ reports speakers bureaus from Roche and BMS, advisory board Celltrion and Norgine. JJ reports grants from Abbvie, Pharmacosmos, Ferring, consulting fees from Abbvie, Boerhinger Ingelheim, BMS, Celltrion, Ferring, Glihead, Janssen Cilag, MSD, Napp Pharma, Novartis,

Orion Pharma, Pfeizer, Pharmacosmos, Takeda, Sandoz, Unimedic Pharma, speakers bureaus Abbvie, Astro Pharma, Boerhinger Ingelheim, BMS, Celltrion, Ferring, Glihead, Hikma, Janssen Cilag, Meda, MSD, Napp Pharma, Novartis, Oriuon Pharma, Pfeizer, Pharmacosmos, Roche, Takeda, Sandoz. TKK reports grants from AbbVie, Amgen, BMS, MSD, Novartis, Pfizer, UCB, consulting fees from AbbVie, Amgen, Biogen, Celltrion, Eli Lilly, Gilead, Mylan, Novartis, Pfizer, Sandoz, Sanofi, speakers bureaus Amgen, Celltrion, Egis, Evapharma, Ewopharma, Hikma, Oktal, Sandoz, Sanofi. LAM reports funding from KG Jebsen foundation, support for infrastructure and biobanking from the University of Oslo and Oslo University Hospital, grants from the Coalition of Epidemic Preparedness Innovations CEPI, speakers bureaus Novartis, Cellgene. GG reports consulting fees from the Norwegian System of Compensation to Patients, AstraZeneca, speakers bureaus Bayer, Sanofi Pasteur, and Thermo Fisher.JTV reports grant from the Coalition of Epidemic Preparedness Innovations (CEPI). FLJ reports grant from the Coalition of Epidemic Preparedness Innovations (CEPI), grant from South-East region Health authority. GLG reports funding from The Karin Fossum foundation, Diakonhjemmet Hospital, Oslo University Hospital, Akershus University Hospital, Trygve Gydtfeldt og frues Foundation, South-East region Health authority, consulting fees AbbVie and Pfizer, speakers fees AbbVie, Pfizer, Sandoz, Orion Pharma, Novartis and UCB, advisory board Pfizer, AbbVie. SWS, IJ, HK, ATT, TTT, JS, SAP, SM, DJW, LSNM, AMA, EBV, GBK, and EAH report nothing to disclose.

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FIGURES

[Figure 1 A-D title]

Humoral response to standard and third vaccine dose

[Figure 1 A-D legend]

A) The violin illustrates the kernel probability density of antibody titres, and the line indicates the median. Dots denote individual patients. Sars-CoV-2 antibodies (anti-RBD) levels in healthy controls and in all patients after standard two dose vaccination, after standard two dose vaccination in patients later receiving a third dose, and after third dose vaccination in patients (n=49). B) Time between last rituximab infusion and first vaccine dose and anti-RBD response in all patients after standard two dose vaccination. No response=anti-RBD<5AU/ml, weak response=anti-RBD 5–70AU/ml and response=anti-RBD≥70AU/ml. C) Anti–receptor-binding domain (RBD) IgG antibody levels measured 2–4 weeks after the second and third vaccine dose. Lines are between the patients' two samples. Horizontal dotted line indicates the cut off for positivity (70 arbitrary units [AU]/mL). D) Time between last rituximab and anti-RBD response after third vaccination (n=49).

[Figure 2 A-C title]

T cell responses after standard and third dose vaccination

[Figure 2 A-C Legend]

A) Quantification of anti-Spike T cell response in patients after the second and third dose of anti-SARS-CoV-2 vaccine and in healthy controls after the second vaccine dose. CD4+ T cell responses (top) and CD8+ T cell responses (below) are shown for all unstimulated/stimulated pairs, Wilcoxon matched-pairs signed rank test is shown with *** and **** for p<0·0001 and p<0.0001 respectively. Positive and negative patients are indicated. B) Analysis of T cell response directed against wild type and delta variant SARS-CoV-2 Spike peptides as in (A)

for patients after second and third vaccine dose (Spearmann correlation). C) Combined data of second and third dose in patients showing response to wild type and delta variant SARS-CoV-2 Spike peptides. Top: percent of specific anti-Spike CD4+ T cells versus anti-Spike CD8+ T cells in responder patients. Below: percent of specific anti-Spike CD4 T cells versus age (y). Spearman correlation is shown. See also appendix p5 for supplementary data for gating and controls.

[Figure 3 title]

Adverse events following standard two dose- and third dose vaccination in patients and controls

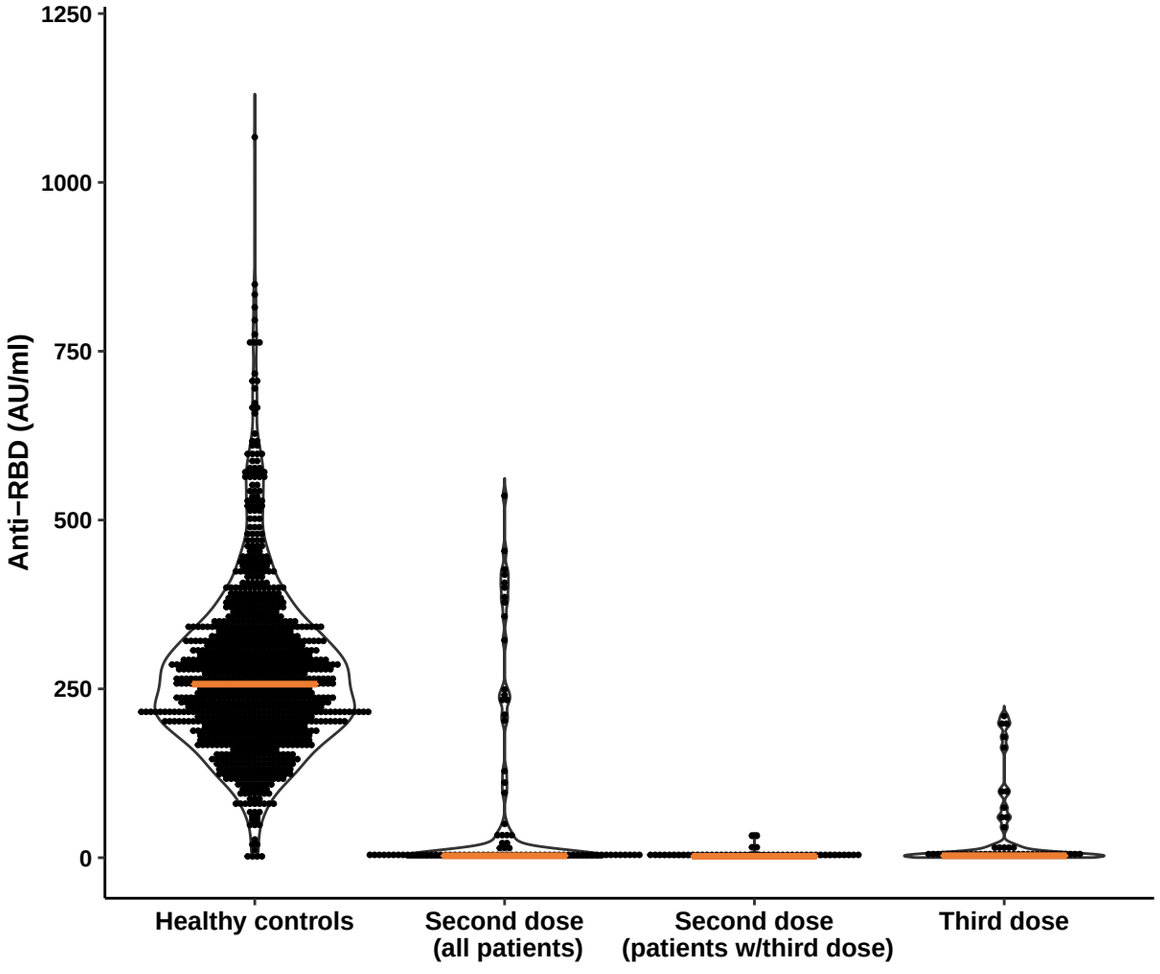
[Figure 3 legend]

Blue, orange and grey bars indicate adverse events reported after the first, second and third vaccine dose, respectively. Adverse events were reported for all patients and a subset (n=246) of healthy controls (health care workers at DH and AHUS).

*Duration not measured

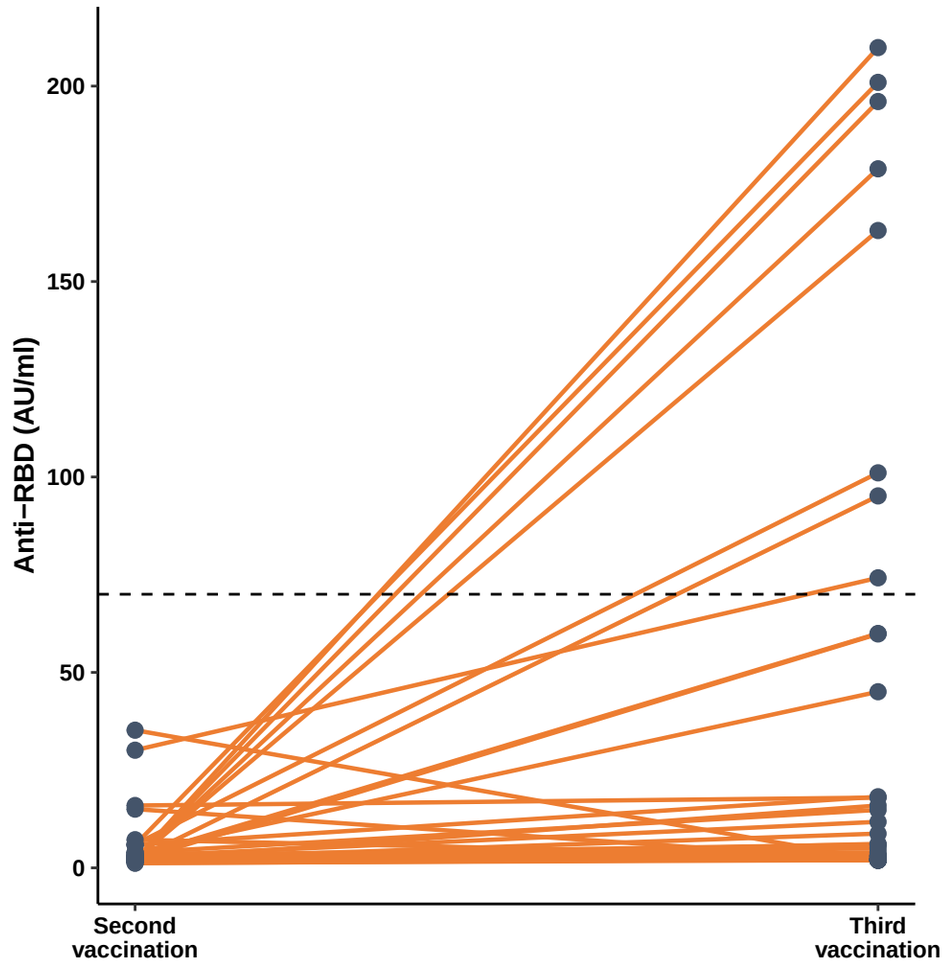
†No patients were hospitalized due to disease flare after vaccinations

A.



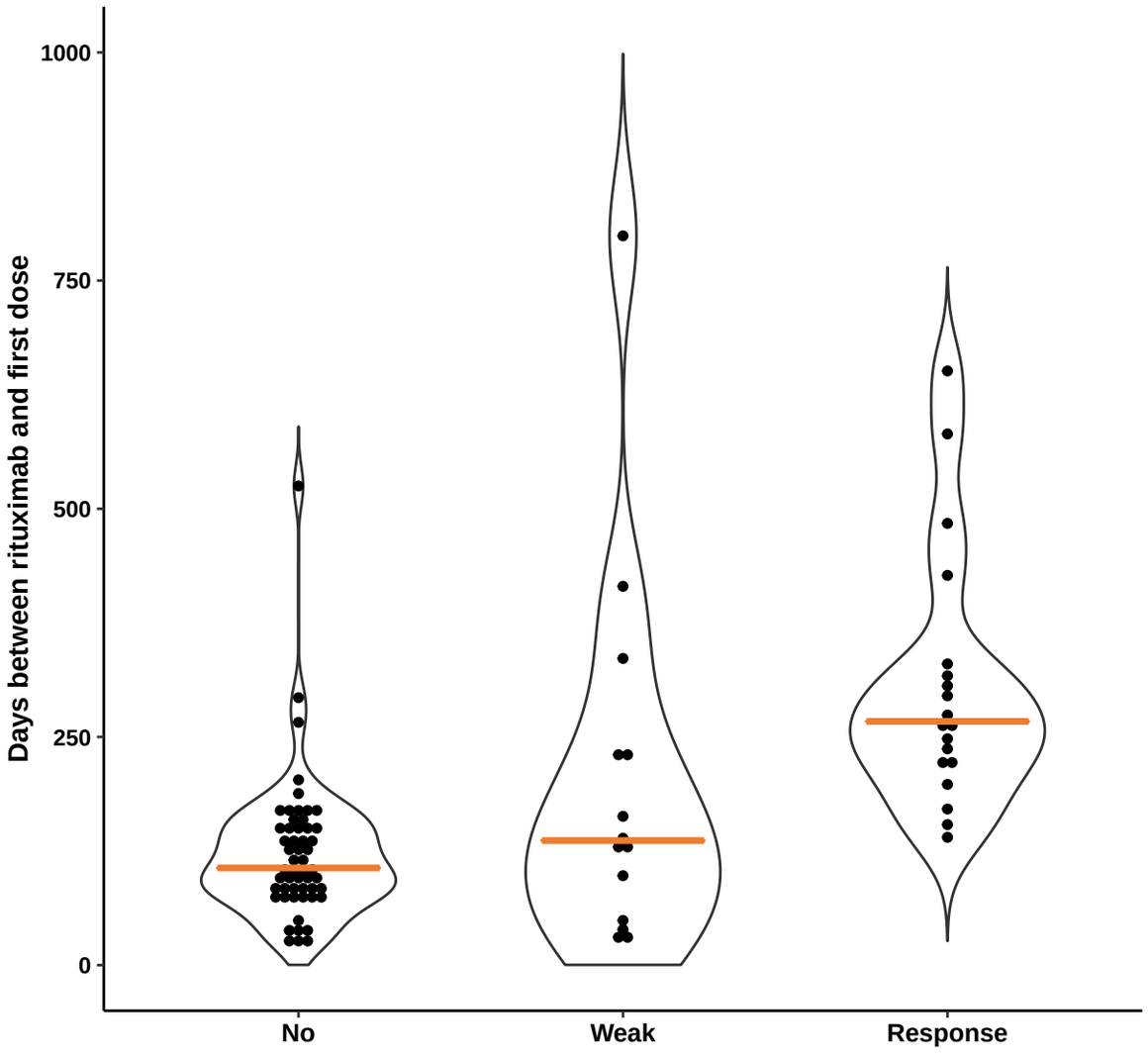
No. observations	1114	87	49	49
Responders (>70)	1096	19	0	8
Median (IQR)	257 (198−327)	3 (2−33)	2 (2−3)	3 (2−18)

C.



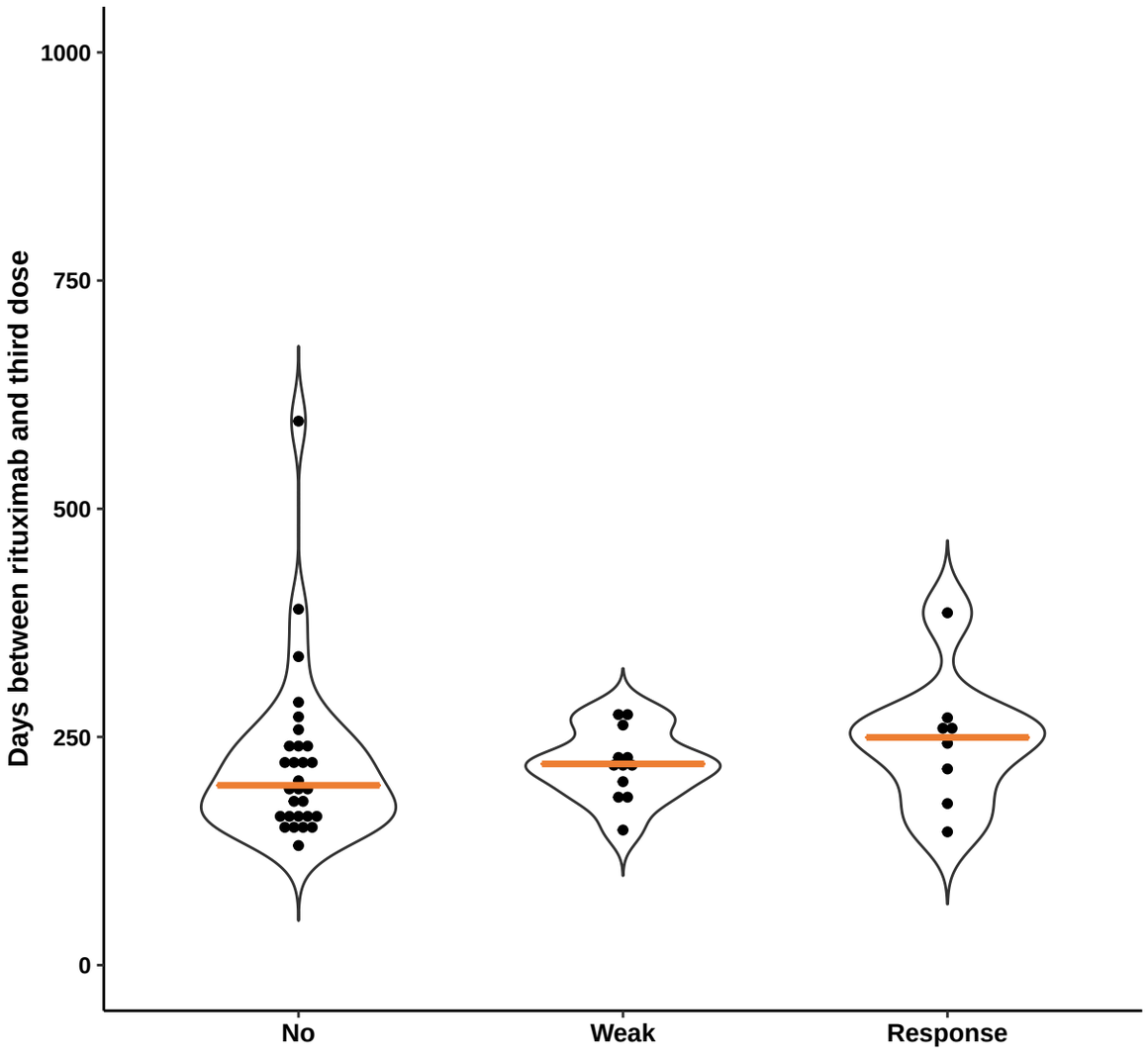
No. observations	49	49
Median (IQR)	2 (2−3)	3 (2−18)
P−value	–	<0·0001

B.

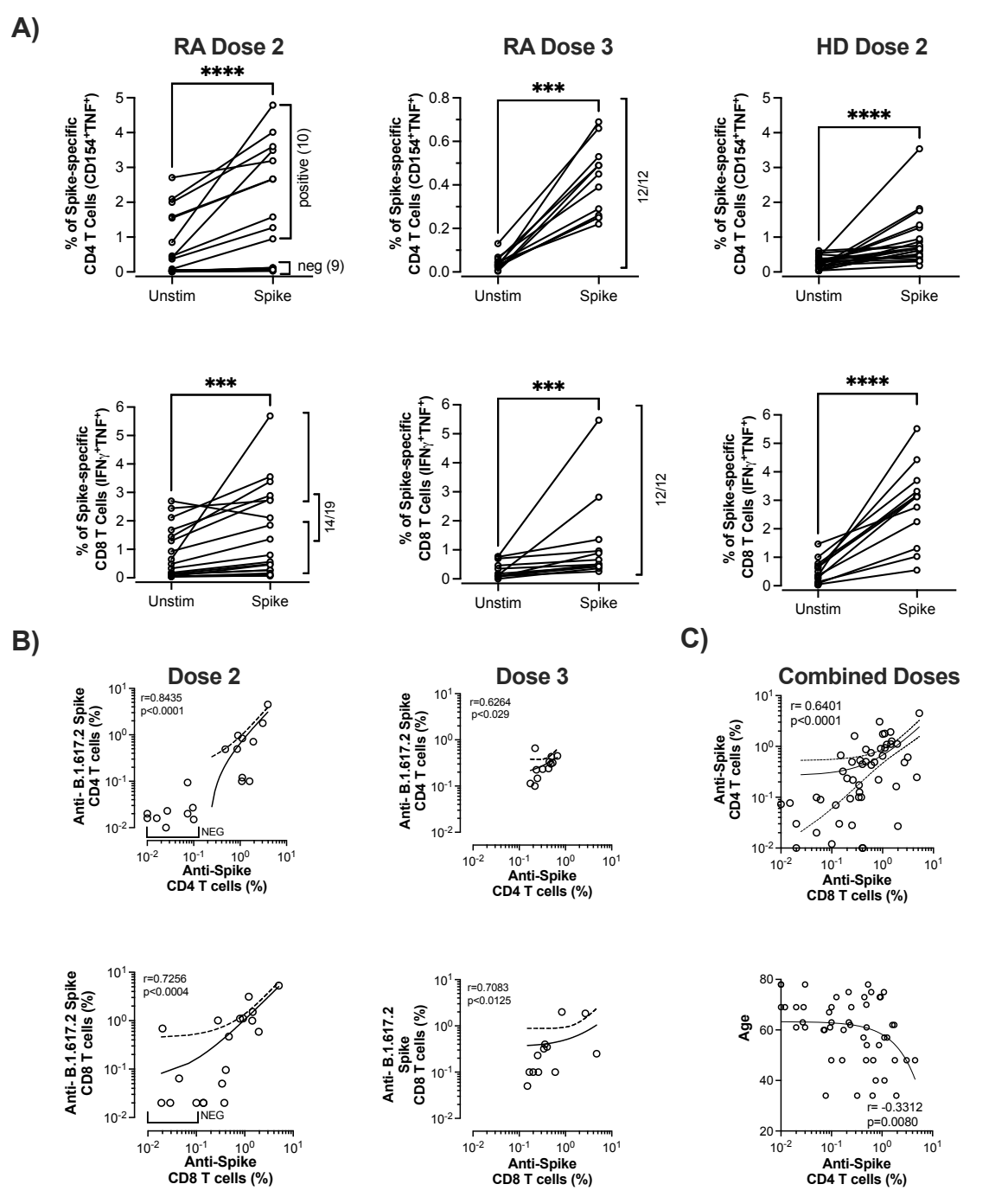


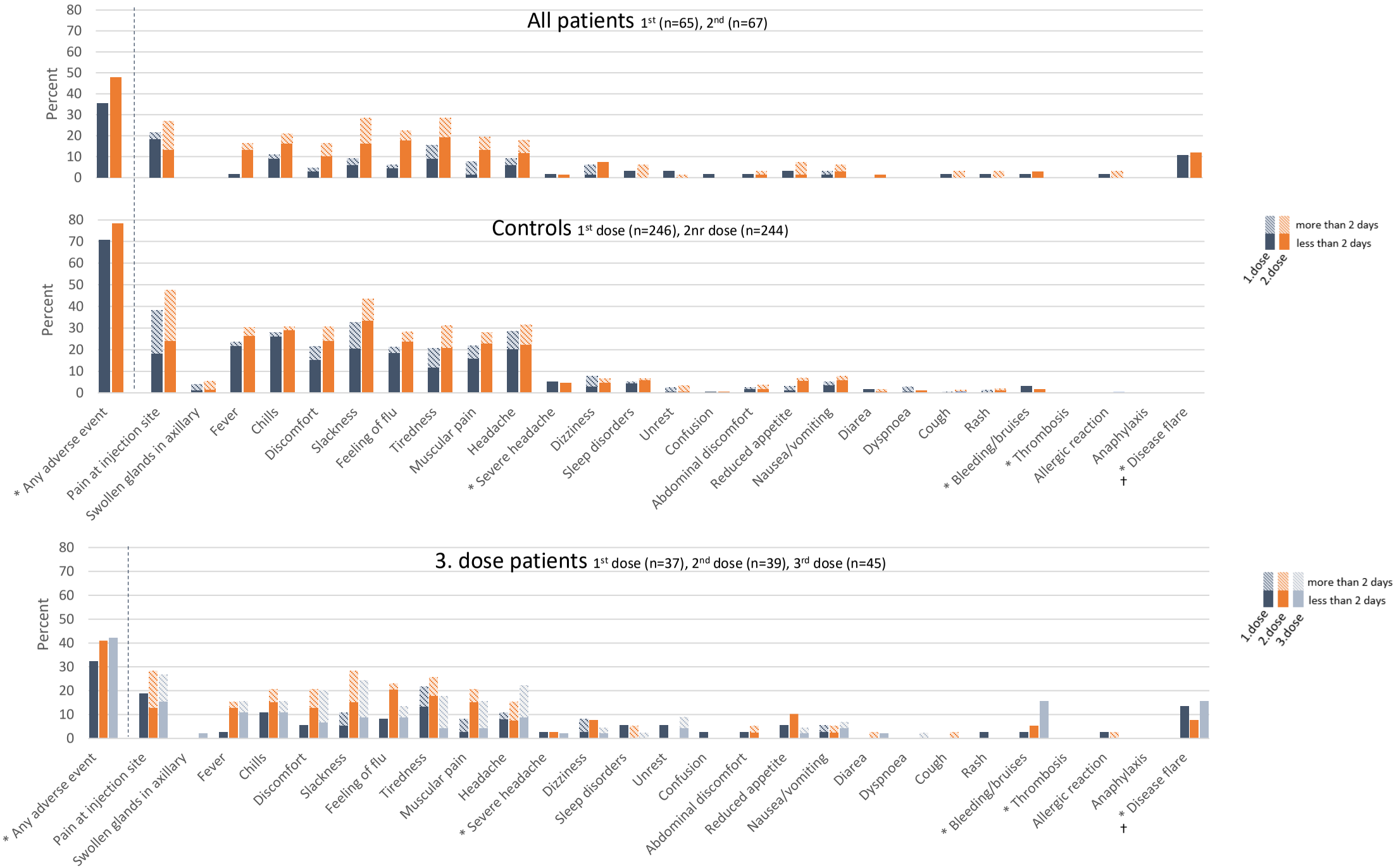
No. observations	54	14	19
Median (IQR)	106 (80−152)	137 (61−233)	267 (222−324)

D.



No. observations	29	12	8
Median (IQR)	197 (163−243)	220 (197−238)	250 (206−265)


A)
RA Dose 2
RA Dose 3
HD Dose 2
****
***
****
% of Spike-specific CD4 T Cells (CD154+TNF+)
positive (10)
neg (9)
12/12
Unstim
Spike
***
***
****
% of Spike-specific CD8 T Cells (IFNγ+TNF+)
14/19
12/12
B)
Dose 2
Dose 3
C)
Combined Doses
r=0.8435
p<0.0001
r=0.6264
p<0.029
r= 0.6401
p<0.0001
Anti- B.1.617.2 Spike CD4 T cells (%)
Anti-Spike CD4 T cells (%)
Anti-Spike CD8 T cells (%)
NEG
r=0.7256
p<0.0004
r=0.7083
p<0.0125
Anti- B.1.617.2 Spike CD8 T cells (%)
Age
r= -0.3312
p=0.0080


All patients 1st (n=65), 2nd (n=67)
Controls 1st dose (n=246), 2nr dose (n=244)
3. dose patients 1st dose (n=37), 2nd dose (n=39), 3rd dose (n=45)
Percent
more than 2 days
less than 2 days
1.dose
2.dose
3.dose
* Any adverse event
Pain at injection site
Swollen glands in axillary
Fever
Chills
Discomfort
Slackness
Feeling of flu
Tiredness
Muscular pain
Headache
* Severe headache
Dizziness
Sleep disorders
Unrest
Confusion
Abdominal discomfort
Reduced appetite
Nausea/vomiting
Diarea
Dyspnoea
Cough
Rash
* Bleeding/bruises
* Thrombosis
Allergic reaction
Anaphylaxis
* Disease flare
†