THE LANCET
Rheumatology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Wieske L, van Dam KPJ , Steenhuis M, et al. Humoral responses after second and third SARS-CoV-2 vaccination in patients with immune-mediated inflammatory disorders on immunosuppressants: a cohort study. *Lancet Rheumatol* 2022; published online March 17. https://doi.org/10.1016/S2665-9913(22)00034-0.

Contents

List of study collaborators	2
Methods	3
Table 1: immunosuppressants per disorder for participants without previous SARS-CoV-2 infection	5
Table 2: immunosuppressants for participants with previous SARS-CoV-2 infections	7
Table 3: Counts of seroconversion in participants without previous SARS-CoV-2 infection after completed standard vaccination	9
Figure 1: antibody neutralisation capacity and immunosuppressants	10
Figure 2: antibody responses per immune-mediated inflammatory disorder	11
Figure 3: antibody responses per immunosuppressants without prior grouping	12
Table 4: characteristics of participants with missing serological assessments	13-14
Figure 4: missing data analysis	15
Table 5: antibody responses per vaccine	16
Table 6: antibody responses per sex	17
Table 7: characteristics for third vaccination groups	18-19
Table 8: counts of seroconversion in participants without previous SARS-CoV-2 infection after third vaccination	20
Table 9: counts of seroconversion after first and second vaccination in hybrid immunity groups and control groups	21
Figure 5: Hybrid humoral responses following SARS-CoV-2 vaccination	22-23
References	24

List of collaborators

Rivka de Jongh - Sanquin, Amsterdam, The Netherlands
Carolien van de Sandt - Sanquin, Amsterdam, The Netherlands
Lisan Kuijper - Sanquin, Amsterdam, The Netherlands
Mariel Duurland - Sanquin, Amsterdam, The Netherlands
Ruth Hagen - Sanquin, Amsterdam, The Netherlands
Jet van den Dijssel - Sanquin, Amsterdam, The Netherlands
Christine Kreher - Sanquin, Amsterdam, The Netherlands
Amelie Bos - Sanquin, Amsterdam, The Netherlands
Viriginia Palomares Cabeza - Sanquin, Amsterdam, The Netherlands
Sergey Nejentsev - Amsterdam UMC, Amsterdam, The Netherlands
Elham Mirfazeli - Amsterdam UMC, Amsterdam, The Netherlands

Methods

Recruiting centres
Participants treated in out-patient clinics at the Amsterdam UMC (locations AMC and VUmc), Erasmus MC Rotterdam, Leiden University Medical Centre, University Medical Centre Groningen, Maastricht University Medical Centre), Utrecht University Medical Centre, and one Rheumatology treatment centre (Reade, Amsterdam Rheumatology & immunology Centre, Amsterdam). Additional participants were recruited from two cohort studies on COVID-19 related disease severity in patients with auto-immune diseases, the ARC, and COMS-19 studies (Trial ID NL8513 and NCT04498286).[1,2]

Methods used to determine participants' sex
Participants were sent an online questionnaire at study entry containing the multiple-choice question "What is your sex?". Answer options were 'female', 'male' and 'not specified'. Participants could only select one option.

Pre-defined immune mediated inflammatory disorders enrolled for this study
Dermatological: atopic dermatitis, psoriasis, pemphigus, other immune-mediated dermatologic conditions
Neurological: multiple sclerosis, neuromyelitis optica spectrum disorder, myasthenia gravis, chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, inflammatory myositis
Rheumatological: rheumatoid arthritis, spondylarthritis, SLE, giant cell arteritis, Sjogren syndrome, vasculitis, other immune-mediated rheumatologic conditions
Gastro-enterological: Crohn's disease, ulcerative colitis, auto-immune hepatitis, other inflammatory bowel disorders

Pre-defined monotherapy treatment groups enrolled for the primary analysis
Before start of the study, we defined the following nine monotherapy groups to be included for the primary analysis: methotrexate, calcineurin inhibitors, purine antagonist, prednisone, TNF-inhibitors, anti-CD20 therapy, ustekinumab, intravenous/subcutaneous immunoglobulin (IVIg/SCIg), and dupilumab. Calcineurin inhibitors were excluded from primary analysis because the number of observations was too low.

Definitions for immunosuppressants, active treatment and treatment grouping
We defined immunosuppressants (ISPs) as either immunosuppressive or immunomodulatory treatment. Active treatment was defined as treatment with a particular ISP in the last three moments prior to the first or third vaccination or if treatment was started between the first and second vaccination (when applicable). For anti-CD20 therapies, cladribine, or cyclophosphamide active treatment was defined as a last administration within 12 months prior to first or third vaccination or between the first and second vaccination. Combination therapies were grouped in the following order: any combination therapy involving anti-CD20 therapies, MMF in combination with corticosteroids, purine antagonists or methotrexate in combination with corticosteroids, purine antagonists or methotrexate in combination with TNF inhibitors, dual combination therapies with corticosteroids and any other ISP, and dual combination therapies with purine antagonists or methotrexate and any other ISP. In patients with corticosteroid monotherapy (n=61), 58 patients received oral prednisone, 2 patients received dexamethasone, and 1 patient received methylprednisolone. For oral prednisone the median dose was 7.5 mg/day (IQR 5–10). For combination therapies, any dose of corticosteroids was used. Hydroxychloroquine was only analysed as monotherapy but not as an adjunctive ISP when used in combination. Monotherapy or combination treatment groups for which less than 10 observations were available were grouped as "others" and were not analysed as separate groups (see Table 1 and 2).

The following treatments were not regarded as systemic immunosuppressants in this study: any topical, inhaled, or rectal administered immunosuppressant, mesalazine, sulfasalazine, and budesonide.

SARS-CoV-2 vaccination
Participants in study were vaccinated according to the Dutch national vaccination campaign. During the course of this study, the ChAdOx1 nCoV-19 (AstraZeneca), BNT162b2 (Pfizer/BioNtech), CX-024414 (Moderna), and Ad.26.COV2.S (Janssen) vaccines were used in this campaign. Participants received their vaccination by any of the health care workers involved in the national vaccination campaign (i.e. municipal health service, general physician, or hospital). National vaccination campaign guidelines including target populations and intervals for the different vaccines differed during the course of this study. Most notably, for people <65 year the target population was changed from ChAdOx1 nCoV-19 (AstraZeneca) to either BNT162b2 (Pfizer/BioNtech) or CX-024414 (Moderna) in May 2021. Also, for healthy individuals with a previous SARS-CoV-2 infection, a second vaccination was made optional. Combining different vaccine types was not yet part of official guidelines during the course of this study. In general, the advisory committee The National Institute for Public Health and the

Environment of the Netherlands advised not to pause or suspend immunosuppressive treatment before or during SARS-CoV-2 vaccination.
In September, the Dutch government decided to implement a third vaccination in the standard induction vaccination scheme for specific vulnerable populations. For patients with immune mediated inflammatory disorders this pertained to patients treated with anti-CD20 therapy, S1P modulators and MMF combination therapies. All patients were invited for vaccination in October/November regardless of the interval between from their second vaccination (in most participants between April-June 2021). The third vaccine in these patients did not replace the booster vaccination which is currently being administered in all patients (December 2021/January 2022). The third vaccinations in our participants were either done by the study team (using CX-024414; Moderna, independent of the first two vaccinations) or by municipal health services (using BNT162b2 (Pfizer/BioNtech) or CX-024414 (Moderna), independent of the first two vaccinations). Especially as part of this study, we also selected subgroups of participants treated with methotrexate, TNF inhibitors, and purine antagonists to receive a third vaccination, which was not standard practice following the national campaign.

From the total study cohort, we selected nine subgroups of participants (total N: 450) for cellular analyses based on their treatment and whether they were previously demonstrated COVID-19 infection for additional blood sampling by venipuncture for storage of serum, plasma and PBMCs. The selected subgroup of participants in this study with additional cellular analysis was vaccinated by the study team with two doses of allocated CX-024414 (Moderna) vaccine with a six-week interval. In participants who received a third vaccination by the study team blood was drawn for cellular analyses at day of vaccination and after 7 days.

Sample size calculation for the primary analysis
Assuming a power of 80%, a p-value of 0·005 (corrected for multiple comparisons), a difference in the proportion of serological responses of 15% (90 to 75%) due to a specific immunosuppressive drug, at least 175 participants per type of immunosuppressive drug are needed.

Data sharing
Aggregated data and code for reproducing the results of our primary analysis can be shared upon reasonable request.

Table 1: Immunosuppressants per immune-mediated inflammatory disorder in participants without SARS-CoV-2 infection

Table showing immunosuppressants in the different immune-mediated inflammatory disorders (IMIDs) during SARS-CoV-2 vaccination (see Methods for definitions of active treatment).

	Rheumatic disorders					**Inflammatory bowel disease**			**Neurological disorders**			**Dermatological disorders**	
No (%)	RA	SpA	SLE	VAS[a]	Other[b]	CD	UC	Other[c]	MS[d]	INP & IMP[e]	MG	AD	Other[f]
	N=224	N=97	N=145	N=69	N=36	N=248	N=143	N=41	N=265	N=134	N=109	N=84	N=163
Controls*	24 (10·7)	18 (18·6)	12 (8·3)	8 (11·6)	7 (19·4)	32 (12·9)	51 (35·7)	4 (9·8)	86 (32·5)	11 (8·2)	40 (36·7)	2 (2·4)	87 (53·4)
a-CD20	6 (2·7)	..	2 (1·4)	15 (21·7)	4 (11·1)	..	..	..	97 (36·6)	2 (1·5)	..	..	2 (1·2)
CS	3 (1·3)	..	10 (6·9)	7 (10·1)	7 (19·4)	..	..	3 (7·3)	1 (0·4)	2 (1·5)	18 (16·5)	..	..
DUP	..	..	..	..	..	..	..	1 (2·4)	..	..	..	55 (65·5)	2 (1·2)
IV/SCIg	..	..	..	..	..	..	..	..	..	79 (59·0)	..	..	..
MTX	71 (31·7)	14 (14·4)	5 (3·5)	1 (1·5)	2 (5·6)	..	..	..	..	5 (3·7)	1 (0·9)	4 (4·8)	25 (15·3)
PA	..	..	14 (9·7)	3 (4·4)	..	46 (18·5)	17 (11·9)	21 (51·2)	1 (0·4)	3 (2·2)	19 (17·4)	..	1 (0·6)
TNF	28 (12·5)	44 (45·4)	..	..	1 (2·8)	97 (39·1)	27 (18·9)	..	..	..	..	..	1 (0·6)
UST	..	..	..	..	..	21 (8·5)	1 (0·7)	..	..	..	..	..	23 (14·1)
S1P mod	..	..	..	..	..	..	..	..	49 (18·5)	..	..	..	..
MMF	..	..	9 (6·2)	1 (1·5)	2 (5·6)	1 (0·4)	..	2 (4·9)	..	1 (0·7)	2 (1·8)	2 (2·4)	3 (1·8)
JAK	..	1 (1·0)	..	..	..	1 (0·4)	25 (17·5)	..	..	..	..	2 (2·4)	..
VED	..	..	..	..	..	20 (8·1)	5 (3·5)	..	..	..	..	..	..
NTZ	..	..	..	..	..	..	..	..	24 (9·1)	..	..	..	..
HCQ	1 (0·4)	..	34 (23·4)	..	1 (2·8)	..	..	..	..	..	..	..	..
CAL	..	..	..	..	..	..	..	..	..	..	..	7 (8·3)	5 (3·1)
a-CD20+ other(s)[1]	7 (3·1)	..	3 (2·1)	16 (23·2)	8 (22·2)	..	..	..	2 (0·8)	8 (6·0)	..	..	3 (1·8)
MMF+CS	..	..	21 (14·5)	1 (1·5)	..	..	..	3 (7·3)	..	6 (4·5)	9 (8·3)	..	..

MTX/PA+ CS	2 (0·9)	..	20 (13·8)	13 (18·8)	2 (5·6)	1 (0·4)	..	4 (9·8)	..	14 (10·4)	11 (10·1)	..	..
MTX/PA+ TNF	45 (20·1)	15 (15·5)	..	..	..	20 (8·1)	9 (6·3)	..	..	..	..	..	5 (3·1)
MTX/PA+ other[2]	11 (4·9)	1 (1·0)	1 (0·7)	..	1 (2·8)	1 (0·4)	1 (0·7)	..	..	..	3 (2·8)	6 (7·1)	2 (1·2)
CS +other[3]	7 (3·1)	1 (1·0)	2 (1·4)	3 (4·4)	1 (2·8)	6 (2·4)	4 (2·8)	2 (4·9)	2 (0·8)	1 (0·7)	3 (2·8)	1 (1·2)	1 (0·6)
other(s)[4]	19 (8·5)	3 (3·1)	12 (8·3)	1 (1·5)	..	2 (0·8)	3 (2·1)	1 (2·4)	3 (1·1)	2 (1·5)	3 (2·8)	5 (6·0)	3 (1·8)

[a]: including small, medium and large vessel vasculitis and other forms of vasculitis except giant cell arteritis;
[b]: including Sjogren's syndrome, giant-cell arteritis, polymyalgia rheumatica and others;
[c]: including auto-immune hepatitis, auto-immune sclerosing cholangitis;
[d]: including 6 patients with neuromyelitis optica spectrum disorder;
[e]: including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and inflammatory myositis;
[f]: including vitiligo, pemphigus, psoriasis and others;
* IMID patients without immunosuppressants
[1] a-CD20 combined with CS 25, MTX 7, PA+CS 3, MTX+CS 2, MMF+CS 2, CAL+CS 1, MMF 1, PA 1, IV/SCIg 1, NTZ 1, MTX+tocilizumab 1, MTX+MMF+CS 1, MTX+cyclophosphamide+CS 1
[2] MTX/PA combined with abatacept 9, DUP 6, CAL 5, tocilizumab 2, UST 1, IL-17A antagonist 1, belimumab 1, IV/SCIg 1, MTX/PA 1
[3] CS combined with TNF 16, CAL 3, VED 2, IV/SCIg 2, belimumab 2, abatacept 2, cyclophosphamide 1, DUP 1, IL-17A antagonist 1, DHODH inhibitor 1, NTZ 1, tocilizumab 1, UST 1
[4]: monotherapies: tocilizumab 6, abatacept 5, IL-17A antagonist 4, belimumab 3, dimethylfumarate 1, glatiramer 1, IL-23 antagonist 1, interferon-beta 1, DHODH inhibitor 1, omalizumab 1
combination therapies: belimumab+MMF 6, CAL+DUP 4, MTX+TNF+CS 5, PA+IV/SCIg+CS 3, PA+CAL+CS 2, JAK+CS+VED 2, MTX+abatacept+CS 1, belimumab+MMF+CS 1, TNF+DHODH inhibitor 1, CAL+MMF 1, MTX+TNF+DHODH inhibitor 1, IV/SCIg+MMF+CS 1, MTX+TNF+CS+VED 1, CS+DHODH inhibitor+tocilizumab 1, MTX+MMF+CS 1, JAK+VED 1, UST+VED 1
IBD: inflammatory bowel disease; RA: rheumatoid arthritis; SpA: spondyloarthritis; SLE: systemic lupus erythematosus; VAS: vasculitis; CD: Crohn's disease; UC: ulcerating colitis; MS: multiple sclerosis; INP & IMP: inflammatory neuropathies and myopathies; MG: myasthenia gravis; AD: atopic dermatitis; a-CD20: anti-CD20 therapy; CS: corticosteroids; DUP: dupilumab; IV/SCIg: intravenous or subcutaneous immunoglobulin; MTX: methotrexate; PA: purine antagonists; TNF: TNF-inhibitors; UST: ustekinumab; S1P mod: sphingosine-1-phosphate receptor modulators; MMF: mycophenolate mofetil; JAK: JAK-inhibitor; VED: vedolizumab; NTZ: natalizumab; HCQ: hydroxychloroquine; CAL: calcineurin inhibitors.

Table 2: Immunosuppressants in participants with previous SARS-CoV-2 infections

Table showing immunosuppressants used in participants with previous SARS-CoV-2 infections (see Methods for definitions of active treatment).

	No (%)
Controls	154 (32·8)
Poor responder immunosuppressants	
a-CD20+other(s)[1]	22 (4·7)
MMF+other(s)[2]	16 (3·4)
S1P mod	9 (1·9)
Other immunosuppressants	
Monotherapies	
TNF	51 (10·9)
MTX	25 (5·3)
DUP	20 (4·3)
PA	19 (4·0)
IV/SCIg	19 (4·0)
CS	16 (3·4)
HCQ	15 (1·1)
NTZ	11 (2·3)
UST	8 (1·7)
CAL	5 (1·1)
JAK	4 (0·9)
VED	3 (0·6)
Combination therapies	
MTX/PA+CS	14 (3·0)
MTX/PA+TNF	22 (4·7)
MTX/PA+other[3]	7 (1·5)
CS + other[4]	7 (1·5)
Other immunosuppressants	
Other(s)[5]	23 (4·9)

[1] a-CD20 monotherapy: 15; combined with other(s): 7 (MTX: 2, IV/SCIg: 1, CS: 6);
[2] MMF monotherapy: 8; combined with other(s): 8 (CS: 8, belimumab: 1);
[3] MTX/PA combined with abatacept: 2, JAK: 1, DUP 1, IV/SCIg: 1, UST: 1, VED: 1;
[4] CS combined with TNF: 4, CAL: 1, cladribine: 1, VED: 1;
[5]: monotherapies: dimethylfumarate: 8, DHODH inhibitor: 1, IL-17A antagonist 2;
combination therapies: DHODH inhibitor+TNF: 1, TNF+VED: 1, PA+belimumab+CS: 1, JAK+CAL+DUP: 1, CAL+DUP: 3, TNF+CAL: 1, CS+IV/SCIg+MTX: 1, TNF+PA+UST: 1, CS+MTX+tocilizumab: 1, JAK+VED: 1.

Total N:470. a-CD20: anti-CD20 therapy; CS: corticosteroids; DUP: dupilumab; IV/SCIg: intravenous or subcutaneous immunoglobulin; MTX: methotrexate; PA: purine antagonists; TNF: TNF-inhibitors; UST:

ustekinumab; S1P mod: sphingosine-1-phosphate receptor modulators; MMF: mycophenolate mofetil; JAK: JAK-inhibitor; VED: vedolizumab; NTZ: natalizumab; HCQ: hydroxychloroquine; CAL: calcineurin inhibitors.

Table 3: Counts of seroconversion in participants without previous SARS-CoV-2 infection after completed standard vaccination

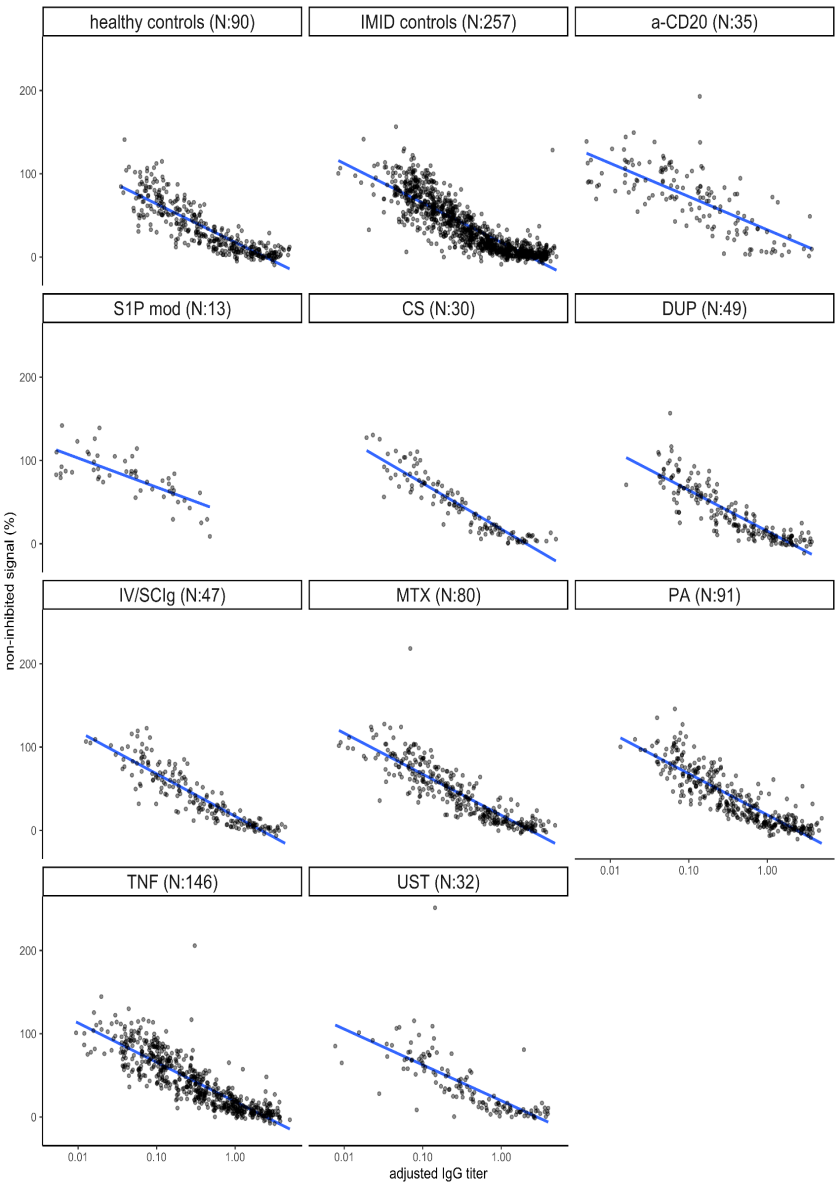
Table showing counts of seroconversion and group size per immunosuppressant in participants without previous SARS-CoV-2 infections after completed standard vaccination from the primary and secondary analysis (see Methods for definitions of active treatment).

	Participants with seroconversion (No, %)
Controls (N=493)	479 (97·2)
Primary analysis	
Monotherapy	
Anti-CD20 therapy (N=128)	39 (30·5)
Corticosteroid (N=51)	48 (94·1)
Dupilumab (N=58)	57 (98·3)
Intravenous or subcutaneous immunoglobulin (N=79)	74 (93·7)
Methotrexate (N=128)	124 (96·9)
Purine antagonist (N=125)	120 (96·0)
TNF inhibitor (N=198)	197 (99·5)
Ustekinumab (N=45)	44 (97·8)
Secondary analysis	
Monotherapy	
Calcineurin inhibitor (N=12)	12 (100)
Hydroxychloroquine (N=36)	35 (97·2)
JAK inhibitor (N=29)	26 (89·7)
MMF (N=23)	19 (82·6)
Natalizumab (N=24)	24 (100)
S1P modulator (N=49)	17 (34·7)
Vedolizumab (N=25)	25 (100)
Combination therapy	
Anti-CD20 + other(s) (N=47)	19 (40·4)
Corticosteroid + other (N=34)	32 (94·1)
MMF + corticosteroid (N=40)	24 (60·0)
MTX or PA + CS (N=67)	58 (86·6)
MTX or PA + other (N=27)	24 (88·9)
MTX or PA + TNF inhibitor (N=94)	88 (93·6)
Other immunosuppressant(s) (N=57)	52 (91·2)

Anti-CD20: anti-CD20 therapy; CS: corticosteroids; MTX: methotrexate; PA: purine antagonists; TNF: TNF-inhibitors; S1P mod: sphingosine-1-phosphate receptor modulators; MMF: mycophenolate mofetil; JAK: JAK-inhibitor.

Figure 1: Antibody neutralisation capacity and immunosuppressants

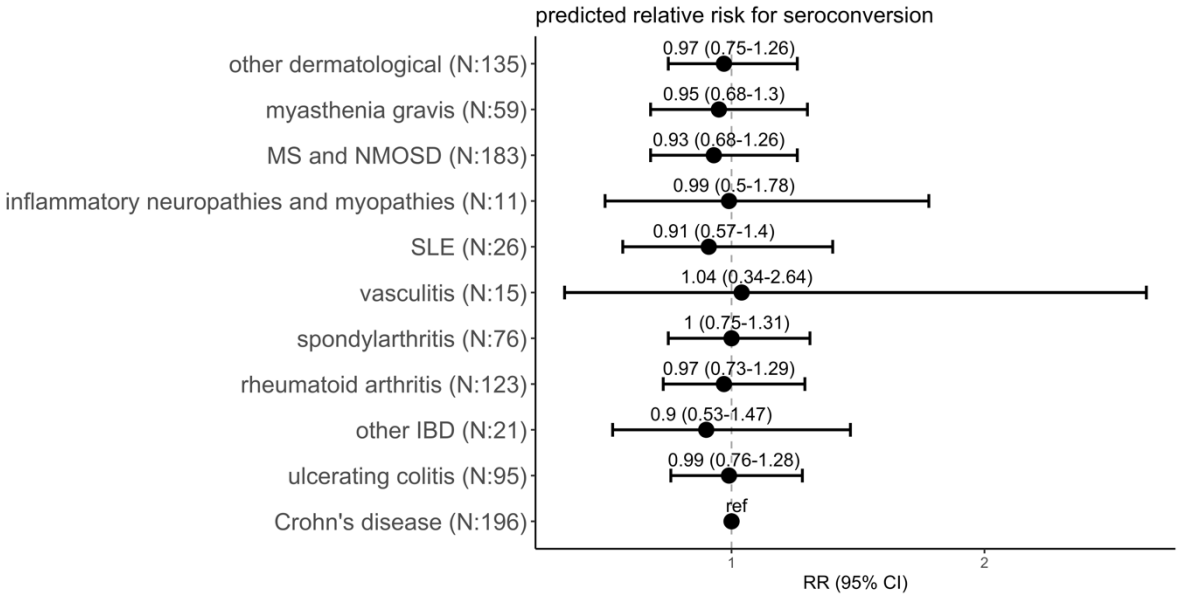
To investigate the capacity of anti-RBD antibodies formed after vaccination to inhibit binding of RBD to the ACE2 receptor, we used an in-house developed competition assay as previously described.[3] In short, in this assay the degree of inhibition of binding of RBD to ACE2 by patient-derived blocking antibodies is measured. This assay is suitable for implementation in large scale studies and correlates with results from the classic viral neutralisation assays.[3] Here we show scatterplots for the different monotherapy groups between anti-RBD IgG titres and non-inhibited signals as measured in the competition assay (expressed as a percentage with 100% indicating no inhibition by anti-RBD antibodies and 0% indicating full inhibition). All samples were tested in a three-fold serial dilution range in four steps. The data from the titration range per sample is plotted; the number of patients tested is indicated for each panel. We selected, where possible random, samples from the larger cohort to have an equal distribution of anti-RBD IgG titres (range from 4 to 632 AU/ml). Only participants without evidence of prior SARS-CoV-2 infection, vaccinated with mRNA vaccines and who reached the threshold of seroconversion were enrolled. For all treatment groups, we observed similar correlations between anti-RBD IgG and non-inhibited signals as to healthy controls and IMID patients without immunosuppressants. This indicates that neutralisation capacity is not influenced by these immunosuppressants.



IMID: immune mediated inflammatory disorders; a-CD20: anti-CD20 therapy; S1P mod: S1P modulators; CS: corticosteroids; DUP: dupilumab; IV/SCIg: intravenous or subcutaneous immunoglobulin; MTX: methotrexate; PA: purine antagonists; TNF: TNF-inhibitors; UST: ustekinumab. Blue lines represent fit lines based on linear regression for visualisation. The y-axis shows the adjusted anti-RBD IgG titre for each dilution tested per sample.

Figure 2: Antibody responses per immune-mediated inflammatory disorder

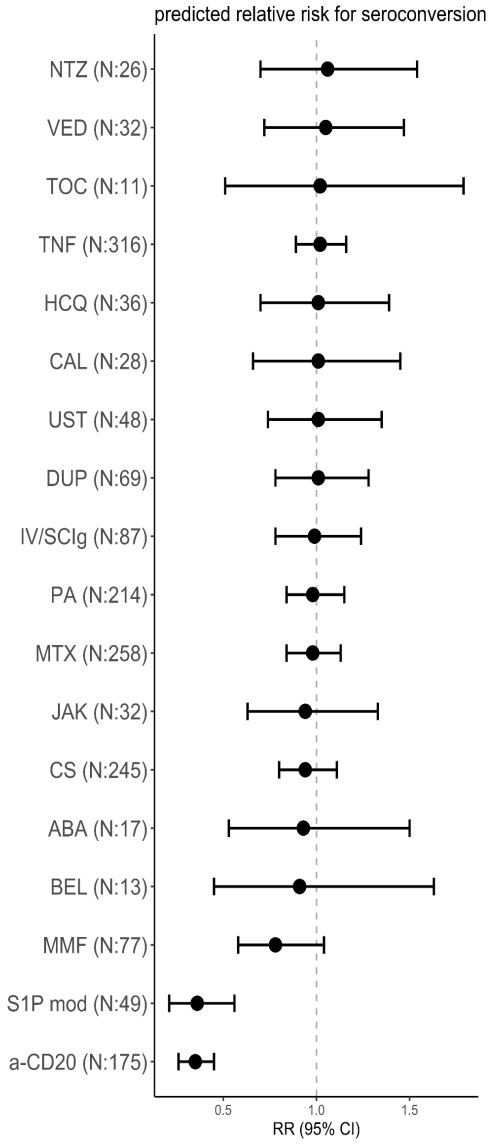
To further investigate antibody response per immune mediated inflammatory disorder (IMID) independent of immunosuppressant, we used a logistic regression model to estimate the relative risk for seroconversion per IMID. For this analysis, we included treatment groups and IMIDs for which at least 2 different IMIDs and with at least 10 observations per IMID were available. Only participants without a previous SARS-CoV-2 infection were included in this analysis. Next to treatment groups, age, sex and vaccine received were added as confounders to the model. Below shows a forest plot with the predicted relative risks for seroconversion for each IMID included in this analysis. None of the IMID was associated with seroconversion.



Treatments included in this analysis are: anti-CD20 therapies, corticosteroids, methotrexate, purine antagonists, TNF-inhibitors, ustekinumab, no immunosuppressant. Total N: 940 patients. RR: relative risk; MS: multiple sclerosis; NMOSD: neuromyelitis optica spectrum disorder; SLE: systemic lupus erythematosus; IBD: inflammatory bowel disease.

Figure 3: Antibody responses per immunosuppressants without prior grouping

To investigate potential influences of our choices in selecting and grouping immunosuppressants for the primary and secondary analysis we used an alternative strategy where we included all immunosuppressants in a logistic regression model. Immunosuppressants were coded as indicator variables. Potential interaction effects between immunosuppressants were studied for pairs and were retained if significant. Immunosuppressants causing convergence problems because too few observations were available were excluded (see legend). Only participants without previous SARS-CoV-2 infections were included in this analysis. Below shows a forest plot with the predicted relative risks for seroconversion for each immunosuppressant included in this analysis. Using this alternative strategy, anti-CD20 therapy and S1P modulators were associated with lower relative risks for seroconversion. The relative risk for MMF was 0.78 (95% CI: 0.58-1.04) and the interaction effect for MMF in combination with corticosteroids was not significant in this analysis.



NTZ: natalizumab; VED: vedolizumab; TOC: tocilizumab; TNF: TNF-inhibitors; HCQ: hydroxychloroquine; CAL: calcineurin inhibitors; UST: ustekinumab; DUP: dupilumab; IV/SCIg: intravenous or subcutaneous immunoglobulin; PA: purine antagonists; MTX: methotrexate; JAK: JAK-inhibitor; CS: corticosteroids; ABA: abatacept; BEL: belimumab; MMF: mycophenolate mofetil; S1P mod: sphingosine-1-phosphate receptor modulators; a-CD20: anti-CD20 therapy. Excluded because of convergence problems: cyclophosphamide; DHODH-inhibitor; dimethylfumarate; glatiramer; IL-17A antagonist; IL-23 antagonist; interferon-beta; omalizumab.

Table 4: Characteristics of participants with missing serology

Table showing clinical characteristics and immunosuppressants for participants eligible for the primary analysis with and without missing serological samples (see figure 1: flowchart).

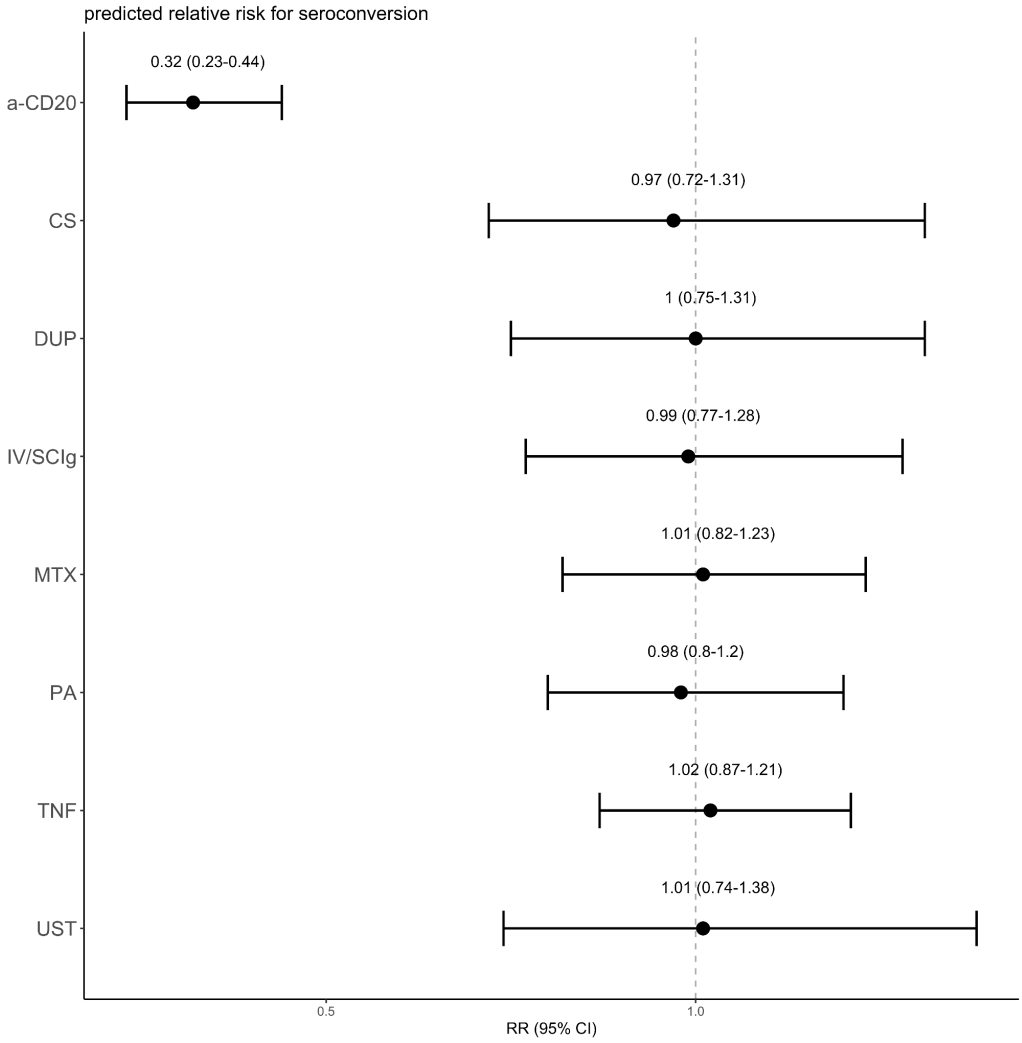
	No previous SARS-CoV-2 infection	
	Complete cases (N=1869)	**Missing serology (N=274)**
Age, mean (SD)	50·7 (13·8)	43·6 (14·3)
Sex, No (%)		
Female	1188 (63·6)	144 (52·6)
Male	681 (36·4)	130 (47·4)
Vaccine received*, No (%)		
mRNA	1606 (85·9)	236 (86·1)
Vector	263 (14·1)	32 (11·7)
Number of immunosuppressants per participant, No (%)		
No immunosuppressants	493 (26·4)	73 (26·6)
1	1034 (55·3)	157 (57·3)
2	312 (16·7)	40 (14·6)
≥3	30 (1·6)	4 (1·5)
Immune-mediated inflammatory disorders, No (%)		
Rheumatic disorders		
Rheumatoid arthritis	224 (12·7)	13 (5·0)
Spondylarthritis	97 (5·5)	7 (2·7)
SLE	145 (8·3)	24 (9·3)
Vasculitis[a]	69 (3·9)	6 (2·3)
Other rheumatic[b]	36 (1·9)	1 (0·4)
Inflammatory bowel disease		
Crohn's disease	248 (14·1)	49 (18·9)
Ulcerating colitis	143 (8·1)	26 (10·0)
Other IBD[c]	41 (2·3)	6 (2·3)
Neurological disorders		
MS and NMOSD[d]	265 (15·1)	35 (13·5)
Inflammatory neuropathies and myopathies[e]	134 (7·6)	18 (7·0)
Myasthenia gravis	109 (6·2)	9 (3·5)
Dermatological disorders		
Atopic dermatitis	84 (4·8)	24 (9·3)
Other dermatological[f]	163 (9·3)	41 (15·8)
Immunosuppressants		
a-CD20	128 (9·3)	17 (8·5)

CS	51 (3·7)	7 (3·5)
DUP	58 (4·2)	20 (10·0)
IV/SCIg	79 (5·7)	6 (3·0)
MTX	128 (9·3)	6 (3·0)
PA	125 (9·1)	13 (6·5)
TNF	198 (14·4)	36 (17·9)
UST	45 (3·3)	13 (6·5)
Other immunosuppressants[g]	564 (41·0)	83 (41·3)

[a]: including small, medium and large vessel vasculitis and other forms of vasculitis except giant cell arteritis;
[b]: including Sjogren's syndrome, giant-cell arteritis, polymyalgia rheumatica and others;
[c]: including auto-immune hepatitis, auto-immune sclerosing cholangitis;
[d]: including 6 patients with neuromyelitis optica spectrum disorder;
[e]: including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and inflammatory myositis;
[f]: including vitiligo, pemphigus, psoriasis and others;
[g]: other immunosuppressants are immunosuppressants not included in the primary analysis (see supplementary methods)
[*]: missing data for six participants missing serology

Figure 4: Missing data analysis

To investigate the effects of missing serological assessments, we repeated the primary analysis on data sets in which missing data was imputed using multivariate imputation by chained equations (10 iterations of 10 imputations). For monotherapy groups included in the primary analysis, 191 participants had missing serological samples that were imputed. Below shows a forest plot with the averaged predicted relative risks for seroconversion for monotherapy groups included in the primary analysis. These estimates did not differ from estimates obtained for the original data set (see figure 2; panel B).



a-CD20: anti-CD20 therapy; CS: corticosteroids; DUP: dupilumab; IV/SCIg: intravenous or subcutaneous immunoglobulin; MTX: methotrexate; PA: purine antagonists; TNF: TNF-inhibitors; UST: ustekinumab

Table 5: Antibody responses per vaccine

Table showing serology results after a completed standard vaccination series in the control group (healthy controls and IMID patients without ISP) without previous SARS-CoV-2 infections for each vaccine. Seroconversion rates and anti-RBD IgG titres were higher in the mRNA vaccines BNT162b2 (Pfizer/BioNTech) and CX-024414 (Moderna) than in the vector vaccines ChAdOx1 nCoV-19 (AstraZeneca) and Ad.26.COV2.S (Janssen).

	BNT162b2 (Pfizer/BioNTech) (N=238)	**CX-024414 (Moderna) (N=188)**	**ChAdOx1 nCoV-19 (AstraZeneca) (N=50)**	**Ad.26.COV2.S (Janssen) (N=17)**
Age, mean (SD)	49·9 (13·3)	50·7 (10·7)	62·6 (7·9)	46·6 (9·8)
Sex, No (%)				
Female	156 (65·5%)	129 (68·6%)	35 (70·0%)	13 (76·5%)
Male	82 (34·5%)	59 (31·4%)	15 (30·0%)	4 (23·5%)
Group				
Healthy controls	52 (21·8%)	51 (27·1%)	2 (4·0%)	6 (35·3%)
Patients without ISP	186 (78·2%)	137 (72·9%)	48 (96·0%)	11 (64·7%)
Seroconversion after completed vaccination				
Yes	236 (99·2%)	187 (99·5%)	44 (88·0%)	12 (70·6%)
No	2 (0·8%)	1 (0·5%)	6 (12·0%)	5 (29·4%)
Anti-RBD IgG titre for participants with seroconversion, median (IQR)	146 [84·2–225]	258 [165–390]	21·0 [11·4–42·1]	9·39 [6·12–17·8]

ISP: immunosuppressants; IQR: interquartile range; RBD: receptor binding domain; SD: standard deviation

Table 5: Antibody responses per sex

Table showing serology results after a completed standard vaccination series in the control group (healthy controls and IMID patients without ISP) without previous SARS-CoV-2 infections by sex. Seroconversion rates and anti-RBD IgG titres did not differ between males and females.

	Female (N=333)	**Male (N=160)**
Age, mean (SD)	50·7 (11·6)	52·9 (13·7)
Group		
Healthy controls	77 (23·1%)	34 (21·3%)
Patients without ISP	256 (76·9%)	126 (78·8%)
Vaccine		
BNT162b2 (Pfizer/BioNTech)	156 (46·8%)	82 (51·3%)
CX-024414 (Moderna)	129 (38·7%)	59 (36·9%)
ChAdOx1 nCoV-19 (AstraZeneca)	35 (10·5%)	15 (9·4%)
Ad.26.COV2.S (Janssen)	13 (3·9%)	4 (2·5%)
Seroconversion after vaccination		
Yes	326 (97·9%)	153 (95·6%)
No	7 (2·1%)	7 (4·4%)
Anti-RBD IgG titre for participants with seroconversion, median (IQR)	170 [76·9–278]	162 [81·6–269]

ISP: immunosuppressants; IQR: interquartile range; RBD: receptor binding domain; SD: standard deviation

Table 7: Characteristics for the third vaccination groups

Table showing participant characteristics in the third vaccination groups

	a-CD20(+others) (N=68)	S1P mod (N=31)	MMF(+others) (N=19)	MTX (N=32)	PA (N=27)	TNF (N=24)
Age, mean (SD)	49·5 (12·5)	45·4 (8·5)	51·6 (16·9)	59·2 (9·8)	38·1 (11·1)	46·8 (13·4)
Sex, No (%)						
Female	47 (69·1%)	19 (61·3%)	13 (68·4%)	26 (81·3%)	14 (51·9%)	8 (33·3%)
Male	21 (30·9%)	12 (38·7%)	6 (31·6%)	6 (18·8%)	13 (48·1%)	16 (66·7%)
Vaccine received, No (%)						
BNT162b2 (Pfizer/BioNTech)	25 (36·8%)	25 (80·6%)	13 (68·4%)	8 (25·0%)	5 (18·5%)	11 (45·8%)
CX-024414 (Moderna)	43 (63·2%)	6 (19·4%)	6 (31·6%)	24 (75·0%)	22 (81·5%)	13 (54·2%)
Time from second to third vaccination, median (IQR)	119 (12–125)	97.0 (91–103)	103 (96–130)	111 (110–113)	110 (106–117)	108 (101–117)
Immune-mediated inflammatory disorders, No (%)						
Rheumatic disorders						
Rheumatoid arthritis	7 (10·3%)	··	··	28 (87·5%)	··	··
Spondylarthritis	··	··	··	3 (9·4%)	··	1 (4·2%)
SLE	3 (4·4%)	··	8 (42·1%)	··	··	··
Vasculitis[a]	14 (20·6%)	··	··	··	··	··
Other rheumatic[b]	4 (5·9%)	··	2 (10·5%)	1 (3·1%)	··	··
Inflammatory bowel disease						
Crohn's disease	··	··	··	··	20 (74·1%)	17 (70·8%)
Ulcerating colitis	··	··	··	··	7 (25·9%)	6 (25·0%)
Other IBD[c]	··	··	1 (5·3%)	··	··	··
Neurological disorders						
MS	37 (54·4%)	31 (100%)	··	··	··	··

Inflammatory neuropathies and myopathies[d]	3 (4·4%)	··	3 (15·8%)	··	··	··
Myasthenia gravis	··	··	5 (26·3%)	··	··	··

[a]: including small, medium and large vessel vasculitis and other forms of vasculitis except giant cell arteritis;
[b]: including Sjogren's syndrome and others;
[c]: including auto-immune hepatitis;
[d]: including chronic inflammatory demyelinating polyneuropathy and inflammatory myositis;

ISP: immunosuppressant; IBD: inflammatory bowel disease; IMID: immune-mediated inflammatory disorder; MS: multiple sclerosis; NMOSD: neuromyelitis optica spectrum disorder; SLE: systemic lupus erythematosus

Table 8: Counts of seroconversion in participants without previous SARS-CoV-2 infection after third vaccination

Table showing counts of seroconversion and group size per immunosuppressant in participants without previous SARS-CoV-2 infections after first, second and third vaccination. Gain of seroconversion is the number of participants without seroconversion after second vaccination but with seroconversion after third vaccination. Loss of seroconversion (i.e. seroreversion), is the number of participants with seroconversion after second vaccination but absent anti-RBD antibodies at the day of third vaccination.

	Seroconversion T1 (No, %)	Seroconversion T2 (No, %)	Seroconversion T3 (No, %)	Missing serum sample T1 (No, %)	Missing serum sample at day of third vaccination (No, %)	Gain of seroconversion after third vaccination (No, %)	Loss of seroconversion at day of third vaccination (No, %)
Controls T1 (N=349)	313 (89·7)	..	..	..	..	..	..
Controls T2 (N=426)	..	423 (99·3)	..	..	..	..	..
Anti-CD20(+others) (N=68)	7 (12·5)	25 (36·8)	31 (45·6)	12 (17·6)	2 (2·9)	13 (19·1)	7 (10·6)
S1P modulator (N=31)	1 (4·4)	11 (35·5)	15 (48·4)	8 (25·8)	1 (3·2)	8 (25·8)	4 (13·3)
MMF(+others) (N=19)	4 (25·0)	10 (52·6)	17 (89·5)	3 (15·8)	2 (10·5)	7 (36·8)	0 (0)
Methotrexate (N=32)	18 (60·0)	30 (93·8)	31 (96·9)	2 (6·3)	0 (0)	1 (3·1)	0 (0)
Purine antagonist (N=27)	22 (91·7)	27 (100)	27 (100)	3 (11·1)	0 (0)	0 (0)	0 (0)
TNF inhibitor (N=24)	17 (70·8)	24 (100)	24 (100)	0 (0)	0 (0)	0 (0)	0 (0)

Anti-CD20: anti-CD20 therapy; MMF: mycophenolate mofetil; S1P: sphingosine-1-phosphate receptor; TNF: tumor necrosis factor alpha; T1: 28 days after first vaccination; T2: 28 days after second vaccination; T3: 28 days after third vaccination

Table 9: Counts of seroconversion after first and second vaccination in hybrid immunity groups and control groups

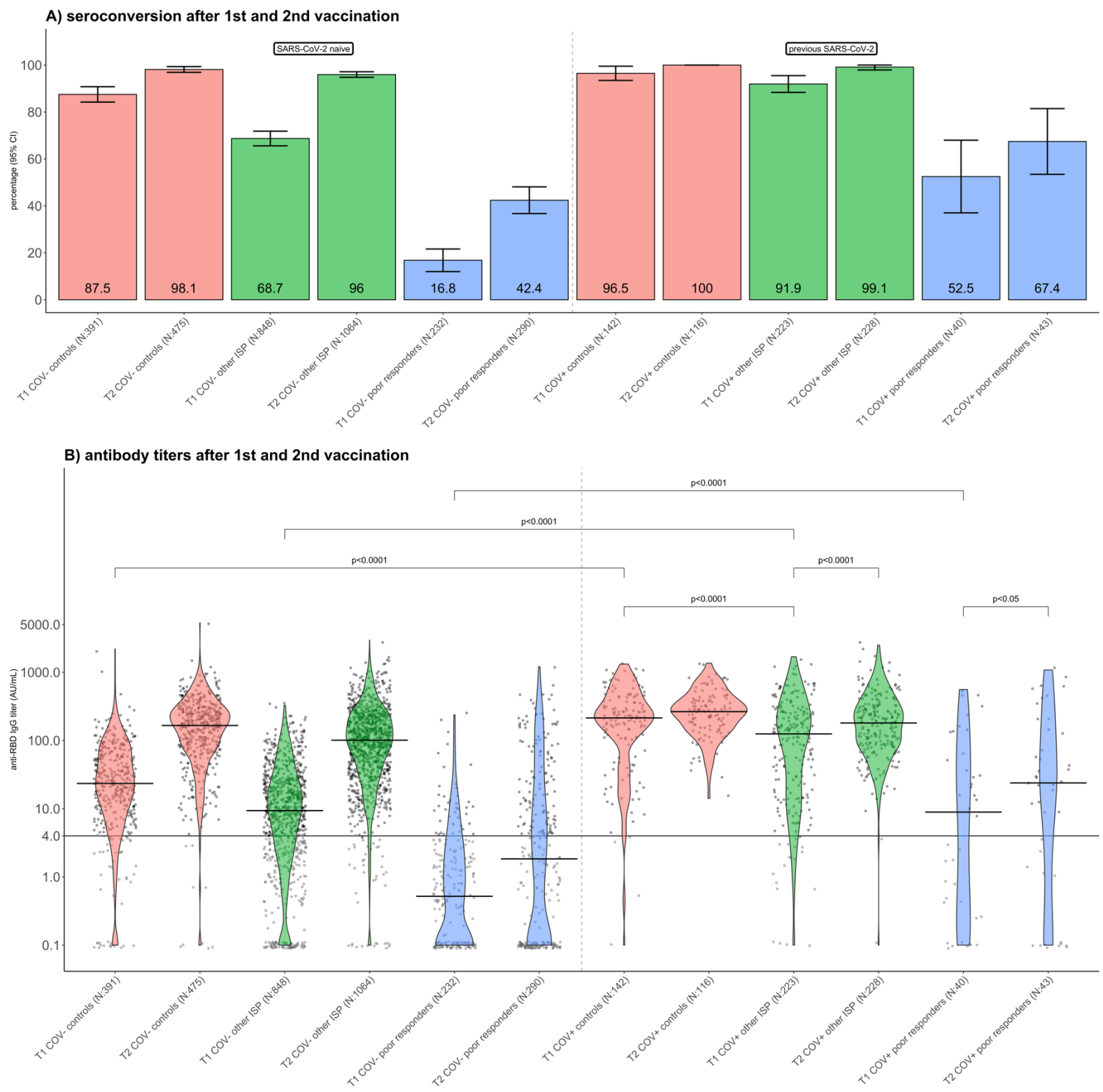
Table showing counts of seroconversion 28 days after first vaccination (T1) and 28 days after second vaccination (T2), in the poor responder immunosuppressants (ie, anti-CD20, S1P modulators, and mycophenolate mofetil combination treatments), other immunosuppressants, and controls (healthy controls and IMID patients without ISP), with or without previous SARS-CoV-2 infection (COV+ and COV-, respectively).

	Seroconversion (No, %)
T1 COV- controls (N=392)	343 (87·5)
T2 COV- controls (N=476)	476 (98·1)
T1 COV+ controls (N=142)	137 (96·5)
T2 COV+ controls (N=116)	116 (100)
T1 COV- other ISP (N=847)	582 (68·7)
T2 COV- other ISP (N=1063)	1020 (96·0)
T1 COV+ other ISP (N=223)	205 (91·9)
T2 COV+ other ISP (N=228)	226 (99·1)
T1 COV- poor responders (N=232)	39 (16·8)
T2 COV- poor responders (N=290)	123 (42·4)
T1 COV+ poor responders (N=40)	21 (52·5)
T2 COV+ poor responders (N=43)	29 (67·4)

Anti-CD20: anti-CD20 therapy; IMID: immune-mediated inflammatory disorder; ISP: immunosuppressant; COV-: without previous SARS-CoV-2 infection; COV+: with previous SARS-CoV-2 infection; S1P: sphinghosine-1-phosphate receptor; T1: 28 days after first vaccination; T2: 28 days after second vaccination

Figure 5: Hybrid humoral responses following SARS-CoV-2 vaccination

Figure showing anti-RBD IgG responses at day 28 after first (T1) and second SARS-CoV-2 vaccination (T2) in participants with (COV+) and without (COV-) a previous SARS-CoV-2 infection. Immunosuppressants (ISP) are grouped into poor responders (i.e. anti-CD20 therapy (monotherapy or in combination), S1P modulators and mycophenolate mofetil (monotherapy or in combination); shown in blue) and other ISP (shown in green). Details on ISP in the "other ISP" group are shown in appendix (pp 7–8). The control group, shown in red, is composed of healthy controls and patients without ISP. Panel A shows the percentage (with 95% confidence interval) of participants with seroconversion (i.e. anti-RBD IgG titre>4 AU/mL) for each timepoint. Panel B shows anti-RBD IgG titres for each timepoint. Grey points indicate titres below the threshold for seroconversion; black points are above this threshold. Horizontal lines indicate the median per group. Comparisons between groups are analysed using a Wilcoxon ranked sum test, comparisons between timepoints are analysed using a paired Wilcoxon signed-rank test.



COV-: without evidence for a previous SARS-CoV-2 infection; COV+: with evidence for a previous SARS-CoV-2 infection; T1: day 28 after first SARS-CoV-2 vaccination; T2: day 28 after second SARS-CoV-2 vaccination; ISP: immunosuppressants

References

1. Boekel L, Steenhuis M, Hooijberg F, et al. Antibody development after COVID-19 vaccination in patients with autoimmune diseases in the Netherlands: a substudy of data from two prospective cohort studies. Lancet Rheumatol 2021;9913(21).
2. Van Kempen ZLE, Strijbis EMM, Al MMCT, et al. SARS-CoV-2 Antibodies in Adult Patients with Multiple Sclerosis in the Amsterdam MS Cohort. JAMA Neurol 2021;78(7):880–2.
3. Steenhuis M, van Mierlo G, Derksen NIL, et al. Dynamics of antibodies to SARS-CoV-2 in convalescent plasma donors. Clin Transl Immunol 2021;10(5).