Full study protocol

Immunity against SARS-CoV-2 in immune-suppressed patients: increased risk of insufficient immunological memory or sufficient protection against re-infection – a Target to B! study

Rationale:

A better understanding of the maintenance of SARS-CoV-2-specific immunity after primo-infection (SIAP) is pertinent to address the risk of re-infection over time, especially for patients with auto-immune disease (AID), including immune-suppressed patients (ISP), which may be at greater risk. In addition to this, there is uncertainty about the efficacy of the much-awaited vaccines in AID patients compared to healthy individuals as it is known for other vaccines that protection is attenuated. A better understanding of SIAP and the effects of induced immunity by vaccination in AID patients is critical to tailor care and guidelines to maximally protect this vulnerable population.

Main hypotheses and study endpoints

Main hypotheses

We hypothesize that:

1) The specific mechanisms of action and level of immunosuppressive medication is the most important determinant of SARS-CoV-2 immunity after vaccination in ISP patients.
2) ISP develop lower titers of SARS-CoV-2 specific antibodies after a primary SARS-CoV-2 infection that show a greater decline over time compared to healthy individuals.
3) ISP develop less SARS-CoV-2 specific T- and B-cells after a primary SARS-CoV-2 infection and SARS-CoV-2 specific T- and B-cells show decreased responses upon ex vivo stimulation, in vitro recall and in vitro reactivation compared to healthy individuals.
4) ISP without previous SARS-CoV-2 infection show an attenuated SARS-CoV-2 specific antibody response upon vaccination compared to patients with AID without systemic immunosuppressive medication and healthy individuals without a previous SARS-CoV-2 infection.
5) ISP with previous SARS-CoV-2 infection show an attenuated SARS-CoV-2 specific antibody response upon vaccination compared to patients with AID without systemic immunosuppressive medication and healthy individuals with a previous SARS-CoV-2 infection.
6) AID patients and healthy controls with a previous SARS-CoV-2 infection show an increased speed of mounting an immune response upon vaccination compared to AID patients and healthy controls without a previous SARS-CoV-2 infection.
7) SARS-CoV-2 (re-) infections and vaccination are risk factors for increases in disease activity in underlying diseases in AID patients and this is related to characteristics and magnitude of the immune response and characteristics of the underlying AID.

Primary endpoints

- Effects of systemic immunosuppressive medication on the serologic response at 28-days after the last SARS-CoV-2 vaccination.
- Difference in SARS-CoV-2-specific B- and T-cell frequencies and functional phenotype and determinants thereof.

Secondary parameters

- Changes in SARS-CoV-2 IgM, IgG and IgA responses over time and determinants thereof.
- Speed of mounting, the magnitude and persistence of the immune response against SARS-CoV-2 and determinants thereof.
- Number of confirmed SARS-CoV-2 (re-) infections and determinants thereof.
- Clinical determinants (including age and gender, disease, disease mechanism and medication) of SIAP.
- Clinical determinants of patient choices and preferences related to vaccine administrations.
- Differences in IgG/IgM/IgA antibodies against different SARS-CoV-2 proteins over time
- Change in disease activity and/or relapses of underlying autoimmune disorders within 8 weeks after SARS-CoV-2 infection and vaccination.
- Changes in and determinants of disease activity and/or relapses in the underlying AID during the study period.
- Incidence and determinations of short-term adverse events after vaccination
- Differences in and determinants of severity of SARS-CoV-2 (re-) infections.
- The number of ISP with SARS-CoV-2 IgM, IgG and IgA antibodies at baseline in patients with previously positive PCR.
- Compare early SIAP development to immunity at follow-up and development of induced immunity after vaccination.

Methods

Study design

We performed a national prospective longitudinal observational multi-arm multicenter cohort study on SARS-CoV-2 vaccinations in selected patients with immune-mediated disorders and specific immunosuppression. This study was approved by the medical ethical committee (NL74974.018.20 and EudraCT 2021-001102-30) and registered at Dutch Trial Register (Trial ID NL8900).

Setting

Participants actively treated in out-patient clinics in six university hospitals (Amsterdam UMC (location AMC), Amsterdam UMC (location VUmc), Erasmus MC, Leiden University Medical Center, University Medical Center Groningen, Maastricht University Medical Center) and one rheumatological treatment center (Reade, Amsterdam Rheumatology & immunology Center, Amsterdam) in the Netherlands were recruited. Additional participants were recruited from two other cohort studies on seroprevalence of SARS-CoV-2 antibodies and COVID-19 related disease severity in patients with auto-immune diseases, the ARC and COMS (Trial ID NL8513 and NCT04498286). Participant were recruited from 16-02-2021 to 20-08-2021.

Participants

Participant were eligible to participate in this study if they were:

1) adults >18 years,
2) were diagnosed with any of the pre-specified immune-mediated disorders
3) were able to understand and complete questionnaires in Dutch.

Participants were recruited as soon as possible to allow baseline sampling prior to first vaccination but could still be included if baseline sampling was no longer possible. Participants were excluded if serum sampling at day 56 after completed SARS-CoV-2 vaccination was no longer possible. Participants with known pregnancy during study entry and concomitant treatment with immunosuppressive medication (like chemotherapy) for cancer or organ-transplantation (including stem-cell transplantation) were also excluded.

During recruitment we actively selected participants with a preceding COVID-19 infection and participants treated with specific forms of immunosuppression to limit heterogeneity of results. We a-priori defined specific monotherapy treatment groups for this selection (table 2). Additionally, we recruited participants treated with other forms of immunosuppressants either as monotherapy or in combinations as used in the pre-specified immune-mediated disorders that were considered clinically relevant (i.e. frequently prescribed and/or based on expected effect on vaccine efficacy).

We recruited patients with the pre-specified immune-mediated disorders and without systemic immunosuppression as disease controls. Additionally, we recruited healthy controls. For healthy controls two additional inclusion criteria were employed: 1) no active or previous autoimmune, oncological or hematological disease and 2) no current or previous treatment with systemic immunosuppressive medication in the last year (last 3 months in case of prednisolone dose of 20 mg per day or less).

From this cohort, we selected 9 subgroups of participants (total N: 450; see table 2) for cellular analyses based on their treatment and whether they were previously demonstrated COVID-19 infection for additional blood sampling by venapuncture for storage of serum, plasma and PBMCs.

SARS-CoV-2 vaccination

Participants in study were vaccinated as part of 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 preceding COVID-19 infection, a second vaccination was made optional. Combining different vaccine types was not yet part of official guidelines during the course of this study. The selected subgroup of participants with additional cellular analysis (n:450) were vaccinated by the study team with two doses of the CX-024414 (Moderna) vaccine with a six-week interval.

For this study, we defined a completed SARS-CoV-2 vaccination as having had two vaccinations of the same type for ChAdOx1 nCoV-19 (AstraZeneca), BNT162b2 (Pfizer/BioNtech) and CX-

024414 (Moderna) vaccines, regardless of the interval, and one vaccination for Ad.26.COV2.S (Janssen) vaccine.

Clinical data collection

Clinical data were collected by the investigators using a standard CRF and by sending online questionnaires to participants.

Participants questionnaires were used to register demographics, patient perceived disease severity and disease activity, any possible COVID-19 infection(s) since start of the pandemic (including date of onset, symptoms and PCR results when performed), hospitalizations for COVID-19 or immune-mediated disease flares. Presence and grade of local and systemic adverse events including any contact with physicians or hospital admission were scored each day for the first 7 days after each vaccination.

Investigators completed CRFs using data from the electronic patient files. CRFs were used to register the immune-mediated diagnosis as noted by the treating physician and dates and dosages for immunosuppressants used starting from 01-01-2020. Hospitalizations reported by participants were verified by investigators and the duration of admission and potential ICU admission were noted. For COVID-19 infections, disease severity was scored as asymptomatic, mild, moderate or severe, based on the WHO disease severity scale. Changes in immunosuppressants during COVID-19 infections were scored. Self-reported immune-mediated disease flares were verified by the investigators, it is recorded if the treating physician also noted a flare and if any changes in treatment were made during the disease flare.

Serum collection

Serum samples were collected using a fingerprick set that was send to participants for home collection at the following timepoints: baseline (prior to first vaccination), day 28 after first vaccination, day 28 after second vaccination (when applicable) and 12 months after first vaccination. Samples were sent at 22 days after vaccination and, at day 28, a reminder email was sent. If participants had not returned their sample at day 30, they were contacted and a new sample was sent, if necessary.

Participants were asked to return the serum tube by mail immediately after acquisition to the central laboratory (Sanquin, Amsterdam, the Netherlands). Upon arrival, the amount of serum was checked and, for samples containing less than 20 microliters of serum, participant was contacted and invited to send a new serum tube.

The baseline sample in the ('cellular') subgroup of participants vaccinated by the study team was acquired by venipuncture. In these patients, serum, plasma and PBMCs were collected at the following timepoints: baseline (directly prior to the first vaccination), day 10 after the first vaccination (only in participants with a preceding COVID-19 infection), the day of second vaccination and day 10 after the second vaccination.

Serological assays

The presence of SARS-CoV-2 specific antibodies were measured in serum or plasma obtained by fingerprick or venipuncture using three assays in the central laboratory. The primary assay used for analyses was an in-house developed anti-RBD IgG ELISA as described previously.[1] Signals were compared to a serially diluted calibrator (arbitrary assigned a value of 100

AU/mL) consisting of pooled convalescent plasma that was included on each plate. Anti-RBD IgG titers were expressed as arbitrary units (AU) per mL (AU/mL).

As sensitivity of the anti- Receptor-Binding Domain (RBD) IgG ELISA was shown to be lower in very low antibody ranges, in selected samples we also used a more sensitive qualitative RBD-Ab (IgG/IgM/IgA) in house developed bridging ELISA, reported as positive or negative, with a previously demonstrated 98.1% sensitivity and 99.5% specificity.[1]

Finally, we used an in-house developed anti-nucleocapsid (N) IgG ELISA to identify asymptomatic SARS-CoV-2 infections in participants in whom a baseline serum sample prior to vaccination was missing and who indicated not to have had a positive PCR for SARS-CoV-2.

Cellular assays

PBMCs were isolated and stored directly after blood withdrawal and broad human immunophenotyping was performed on a selection of the whole blood samples. The number and/or phenotype of COVID-specific T and B cells were determined in the stored PBMC samples by ELIspot or by flowcytometry-based approaches. Antigen-specific B cells were visualized by flowcytometry using tetramerized and fluorescently labelled antigens. Several antigens were studied eg. Spike, RBD, N and control antigens such as tetanus toxoid and influenza HA. Antigen-specific T cells were identified by upregulation of activation markers during overnight stimulation in activation induced marker (AIM) assays.[2] For both the T and B cell analysis a big marker panel was included to deep-phenotype the antigen-specific cells.

Outcome definitions

For each type of immunosuppression, we defined what constitutes active treatment. For anti-CD20 therapy, we discerned two forms, i.e. current or previous treatment, to discern short- and long-term effects. Treatment groups were created for every (combination of) immunosuppressant that met the definition of active treatment at the moment of first vaccination.

Seroconversion after vaccination for the primary analysis was defined as a binary outcome using results from the anti-RBD IgG ELISA and RBD-Ab bridge ELISA. Seropositive was defined as samples >4 AU/mL in the anti-RBD IgG ELISA or a positive result in the RBD-Ab bridge ELISA, when assessed. A cutoff of 4 AU/mL represents 99% specificity in pre-outbreak sera.[3] Seronegative was defined as a negative result on the anti-RBD IgG ELISA and a negative result in the RBD-Ab bridge ELISA, when assessed. For secondary analyses, we used a 4-fold increase in anti-RBD IgG titers between baseline and day 28 after completed vaccination as an additional definition of seroconversion.

A previous SARS-CoV-2 infection was defined as a self-reported positive PCR for SARS-CoV-2 at any time preceding, during or after vaccination up to day 28 after the last SARS-CoV-2 vaccination and/or evidence of anti-RBD antibodies at baseline as assessed with any of the two assays and/or evidence of anti-N IgG antibodies in the sample obtained in day 28 after completed vaccination.

Statistical analysis plan for the primary outcome

The primary outcome for this study was a per-protocol analysis of the adjusted relative risk (ARR), shown with 95% confidence interval adjusted for multiple comparisons (95% CI) and corrected for age and vaccine type, for seroconversion in the predefined primary monotherapy treatment groups at day 28 after completed SARS-CoV-2 vaccination in participants without previous SARS-CoV-2 infection as compared to the control group of participants without immunosuppressive treatment and healthy controls.

As secondary outcome, we investigated the adjusted association, reported with the regression coefficient and 95% CI and corrected for age and vaccine type, separately for participants with or without a previous SARS-CoV-2 infection, between all treatment groups and the log transformed anti-RBD IgG titer at day 28 and the change in anti-RBD IgG titer between baseline and day 28 when available compared to the control group.

For participants in whom a baseline sample was available, we calculated the adjusted relative risk, corrected for age and vaccine type and separately for participants with or without a previous SARS-CoV-2 infection, for seroconversion based on a 4-fold increase in anti-RBD titer for all treatment groups as compared the control group. We also calculated the adjusted relative risk, corrected for age and vaccine type and separately for participants with or without a previous SARS-CoV-2 infection, for seroconversion for all treatment groups as compared the control group.

Sample size calculation for the primary outcome

We a-priori defined a difference in the proportion of seroconversion of at least 15% between a treatment group and controls to be clinically meaningful based on previous studies on immunosuppression in other vaccinations.[4] Before start of the study, the aim was to study 10 primary monotherapy groups in the primary analysis, yielding a sample size of 175 for each primary monotherapy assuming a 15% difference in seroconversion (90 to 75%) compared controls and an adjusted p-value of 0.005 for 10 comparisons.

After start of recruitment, we observed that recruitment for some of the primary monotherapy groups lagged and other studies reported much larger effects of certain immunosuppressive treatments on seroconversion than 15%.[4] Therefore, we decided to adapt to sample size calculation to a step-wise increasing model. Each monotherapy treatment group surpassing a minimum of 50 participants were included in the primary analysis and for each added comparison the 95% confidence interval was adjusted accordingly.

Literature

[1] Vogelzang EH, Loeff FC, Derksen NIL, et al. Development of a SARS-CoV-2 total antibody assay and the dynamics of antibody response over time in hospitalized and nonhospitalized patients with COVID-19. *J Immunol.* 2020;205(12):3491-3499. doi: 10.4049/jimmunol.2000767.

[2] Oja AE, Saris A, Ghandour CA, et al. Divergent SARS-CoV-2 specific T- and B-cell responses in severe but not mild COVID-19 patients. *Eur J Immunol*. 2020;50(12):1998-2012. doi:10.1002/eji.202048908

[3] Steenhuis M, van Mierlo G, Derksen NI, et al. Dynamics of antibodies to SARS-CoV-2 in convalescent plasma donors. *Clin Transl Immunology*, 2021;10(5) doi: 10.1002/cti2.1285.

[4] Nguyen DL, Nguyen ET, Bechtold ML. Effect of immunosuppressive therapies for the treatment of inflammatory bowel disease on response to routine vaccinations: A meta-analysis. *Dig Dis Sci.* 2015;60(8):2446-53. doi: 10.1007/s10620-015-3631-y.

Appendix

Table 1: pre-defined immune mediated disorders

immune-mediated disorder
Atopic dermatitis
Other immune-mediated dermatologic conditions
Pemphigus
Psoriasis
Auto-immune hepatitis
Crohn's disease
Ulcerative colitis
Chronic inflammatory demyelinating polyneuropathy
Myasthenia gravis
Multifocal motor neuropathy
Myositis
Multiple Sclerosis
Neuromyelitis optics
SLE
Giant cell arteriitis
Rheumatoid arthritis
Sjogren syndrome
Spondylarthritis
vasculitis

Table 2: pre-specified treatment groups

primary monotherapy groups		cellular analysis disease group*
	methotrexate	rheumatoid arthritis
	ciclosporine	
	thiopurine/ azathioprine	inflammatory bowel disease
	prednison/ prednisolone	
	TNF-alpha inhibitors	inflammatory bowel disease
	ocrelizumab/ rituximab	multiple Sclerosis
	ustekinumab	
	IVIg/SCIg	
	dupilumab	
	mycofenolate mofetil	
other monotherapy groups	JAK-inhibitors	rheumatoid arthritis
	abatacept	rheumatoid arthritis
	tocilizumab	
	natalizumab	
	fingolimod	
	vedolizumab	
combination therapy groups	azathioprine + prednisone	
	thiopurine + TNF-alpha inhibitors	
	methotrexate + TNF-alpha inhibitors	
	methotrexate + prednisone	
	rituximab + others	

* for cellular analyses the following additional groups were recruited: multiple Sclerosis, inflammatory bowel disease and rheumatoid arthritis without systemic immunosuppression, multiple Sclerosis, inflammatory bowel disease and rheumatoid arthritis with a preceding COVID-19 infection and healthy controls with or without a preceding COVID-19 infection.