WP4 Deliverable 4.4

Quality and kinetic of long-term immune response to COVID-19 vaccination in fragile patients

Alma Mater Studiorum – Università di Bologna (UNIBO)

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Executive summary

This document is the deliverable *"D4.4 – Quality and kinetic of long-term immune response to COVID-19 vaccination in fragile patients"* of the European project *"ORCHESTRA – Connecting European Cohorts to Increase Common and Effective response to SARS-CoV-2 Pandemic: ORCHESTRA"*.

This document has been produced for the European Commission as report on the quality and kinetic of long-term immune response to COVID-19 vaccination in fragile populations.

The report summarizes the antibody response levels at multiple timepoints since first vaccination dosage until 6 months after first booster dosage in different cohorts of adult fragile individuals including solid organ transplanted (SOT) recipients, persons living with HIV infection, patients with hematological and oncological disease, cystic fibrosis and Parkinson disease.

The results on quality and kinetic of serological response to COVID-19 vaccination in the pediatric population are reported in a separated dedicated section of this report.

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Background

The introduction of vaccination for SARS-CoV2 has changed the course of COVID-19 pandemic. However, in some settings as in immunocompromised hosts the effectiveness of vaccination has been shown to be lower than in general population (1,2). This has been correlated with a lower immunological, generally serological, response (3). Indeed, within the ORCHESTRA WP4 prospective study, we showed that the antibody response (AbR) rate was lower in SOT compared to healthcare workers (HCW). AbR levels increased slowly in SOT, reaching a plateau several weeks after vaccination onset, and started weaning soon after (4). In a further analysis, we observed that the probability of AbR was lower in some types of SOT recipients (as heart transplant recipients), and this correlated with an increased risk of developing breakthrough infections (BI) (5). Finally, we have shown that using clinical data is difficult to predict the AbR after booster dosage (6). These findings could be useful to improve preventive strategies in a specific fragile setting, such as that of SOT recipients, but not for prioritizing among fragile populations those at highest risk of having lower immunological response, thus potentially at higher risk of developing BI.

The aim of this study is to assess long-term AbR after at least one booster dosage of SARS-CoV2 mRNA vaccine in different types of fragile populations included in WP4 as SOT recipients, persons living with HIV, patients with hematological malignancy, patients with solid tumor, patients with cystic fibrosis and those with Parkinson's disease. Data on serological response to SARS-CoV2 vaccination in pediatric patients will be also provided as an Annex. We deem these findings could be useful to prioritize fragile patients needing preventive strategies (i.e., serological screening, booster dosage, administration of monoclonal antibodies).

Methods

Study design, setting and population

Multicenter prospective longitudinal cohort within the Horizon 2020 ORCHESTRA project (https://orchestra-cohort.eu/) which aims to create a new pan-European cohort to rapidly advance the knowledge on the COVID-19 infection. The study was approved by the Agenzia Italiana del Farmaco (AIFA) and the Ethics Committee of Istituto Nazionale per le Malattie Infettive (INMI) Lazzaro Spallanzani (document n. 359 of Study's Registry 2020/2021) and

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registered at ClinicalTrials.gov with the number NCT05222139. Informed consent was obtained from all the enrolled patients.

The cohort runs at 32 hospitals (26 in Italy including UNIBO-Bologna, UNIVR-Verona, Padova, Vicenza, Treviso and 21 HIV specialized clinical centers belonging to the Italian HIV network of the ICONA Foundation; 4 in Spain - SAS-Seville, EPICO-Madrid and Valencia - one in Luxembourg – LIH - and one in Argentina – UBA-Buenos Aires). Participants were enrolled from March 1st, 2021 to December 31st, 2021 and followed-up until December 31st 2022. The database was locked on June 30th 2023 after careful revision for incongruent or missing data. Data sources were clinical charts and hospital electronic records. All data were gathered anonymously and managed using REDCap electronic data capture tools hosted at the Interuniversity Consortium CINECA (https://redcap.orchestra.cineca.it/) (7).

Adult fragile patients undergoing SARS-CoV-2 vaccination during the enrolment period who accepted to participate into the ORCHESTRA project were prospectively enrolled. As previously described (4) (6), patients were assessed for AbR to SARS-CoV-2 vaccination at pre-defined timepoints: first dose (t0), second dose (t1), 3±1 month after the first dose (t2), at 1 month after the third dose (t3), at 6 months after the third dose (t4). All patients had a minimum follow-up of six months after the third dosage.

Variables

Primary endpoint was AbR at t4. The response was stratified into non-reactive (<5.58 BAU/mL), inconclusive (5.58-<45 BAU/mL), positive-low (45-<205 BAU/mL), positive-mild (205-<817 BAU/mL), and positive-high (>817 BAU/mL) according to WHO International SARS-CoV-2 Antibody Standards criteria. For the purpose of the study, negative AbR was defined as an anti-receptor binding domain (RBD) titer <45 BAU/mL (including non-reactive and inconclusive results). Exposure variables collected at t0 included age, sex, comorbidities according to the Charlson index; **for solid organ transplant recipients:** type and date of transplant, immunosuppressive regimen, receipt of induction regimen in the past 6 months, and graft function (defined as good, impaired or failure according to the judgement of attending physicians) were collected at each timepoint; **for hematological patients:** date of diagnosis, type of disease, stage at diagnosis, disease stage at transplantation, time from diagnosis to HSCT, characteristics of HSCT, development and characteristics of GVHD; **for persons living with HIV:** risk category, date of HIV diagnosis, previous AIDS diagnosis, date of first ARV start, CD4+ T cell count Nadir (cells/mm^3) and date, HIV-RNA zenith (copies/mL) and date; **for**

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cancer patients: cancer for which it is being treated, primary site, histology, date of diagnosis, mutations detected, PD-L1 expression (% or NA), stage at diagnosis, baseline stage, number and type of metastatic sites; **for PD patients:** date of Parkinson's disease diagnosis, disease worsening during the pandemic; **for cystic fibrosis patients:** date of diagnosis, concomitant pathologies, bi-pulmonary transplant; **for rheumatological patients:** type of rheumatological disease, date of diagnosis, disease evolution, pulmonary rheumatological disease.

Laboratory assays

Detection of AbR was performed with Elecsys® Anti-SARS-CoV-2 ECLIA assay and V-PLEX SARS CoV-2 Panel 6 Kit (IgG) from Meso Scale Discovery (MSD, MD, USA) according to the manufacturer instructions and as previously described (4).

Statistical analysis

To initiate a comprehensive evaluation of long-term antibody response (AbR) distributions following at least one booster dosage of the mRNA SARS-CoV-2 vaccine among distinct fragile populations, the stratified WHO International SARS-CoV-2 Antibody Standards criteria were employed. These criteria classified antibody levels into five ordinal groups: non-reactive, inconclusive, positive-low, positive-mild, and high. At each data collection time point (t0 - t4), the count of individuals within each of these groups was calculated for every fragile population (HIV, Oncological/Hematological, Parkinson, and Solid Organ Transplant recipients).

For comparing these frequency distributions across fragile population groups at each time point, the relative frequencies for each category were computed. This process involved dividing the number of individuals within each category of a specific group by the sum of individuals across all five categories within that same group. These resultant frequencies provided an estimate of the unconditional distribution across groups for the respective fragile population.

Equipped with these distribution estimates, a two-sample Chi-Square test was conducted, assuming a null hypothesis of no differences. The aim was to determine whether a statistically significant variance exists in antibody response distributions across fragile population groups for a specific time point. P-values were utilized to assess significance. This analytical process was replicated for all five time points. Individuals lacking reported antibody levels at any time point (t0-t4) were excluded from calculations involving this time point. However, they were included for time points with available observations.

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For a deeper exploration of long-term AbR distributions, the categories were consolidated into three groups: non-reactive (encompassing non-reactive and inconclusive), mild (including positive-low and positive-mild), and high (referring to high). Transition probabilities from one time point to the next were computed for each fragile population group and category. This entailed determining the proportions of individuals transitioning to each category at the subsequent time point. A practical example clarifies this process. To ascertain the transition probability of SOT patients shifting from non-reactive to mild at t0, the count of SOT patients displaying a non-reactive outcome at t0 was tallied. Subsequently, the count of these same individuals exhibiting a mild reaction at t1 was counted. The number of mild reactions was then divided by the total count of non-reactive results at t0, yielding the transition probability. Observations without records at the following time point (t1 in the previous example) were omitted. These transition probabilities were subsequently utilized in paired t-tests to compute statistical disparities across groups and time points. Significance was determined by the means of p-values.

Results

The study cohort consisted of 1817 SOT recipients (908 kidney, 441 liver, 408 heart, 60 lung), 1817 onco-hematological patients (1440 solid cancer, 377 hematological malignancy), 400 persons living with HIV, 245 patients with cystic fibrosis and 156 patients with Parkinson's disease (PD). All patients received the first two doses with BNT162b2 (n=2796, 81.1%) or mRNA-1273 (n=430, 12.5%) vaccine, with some patients showing missing information for the vaccine manufacturer (n=222, 6.4%). Overall, 2606/4421 (59%) patients received a third dosage with BNT162b2 (n=1269, 48.7%) or mRNA-1273 (n=873, 33.5%), with some patients also showing missing values (n=464, 17.8%). All patients started AbR monitoring at the administration of first vaccine dosage except those with PD in whom the monitoring started at booster dosage.

For a detailed examination of all results across the groups, we have developed an interactive online dashboard, which can be used as illustrated in Figures 1 and 2.

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Figure 1: Interactive Shiny application interface showcasing the distribution of antibody levels across different fragile patient groups and time points after COVID-19 vaccination. Access to the app via: https://apps-carolina2107.shinyapps.io/shiny/ ; username: shiny-orchestra ; password: HFNSGHRI759261

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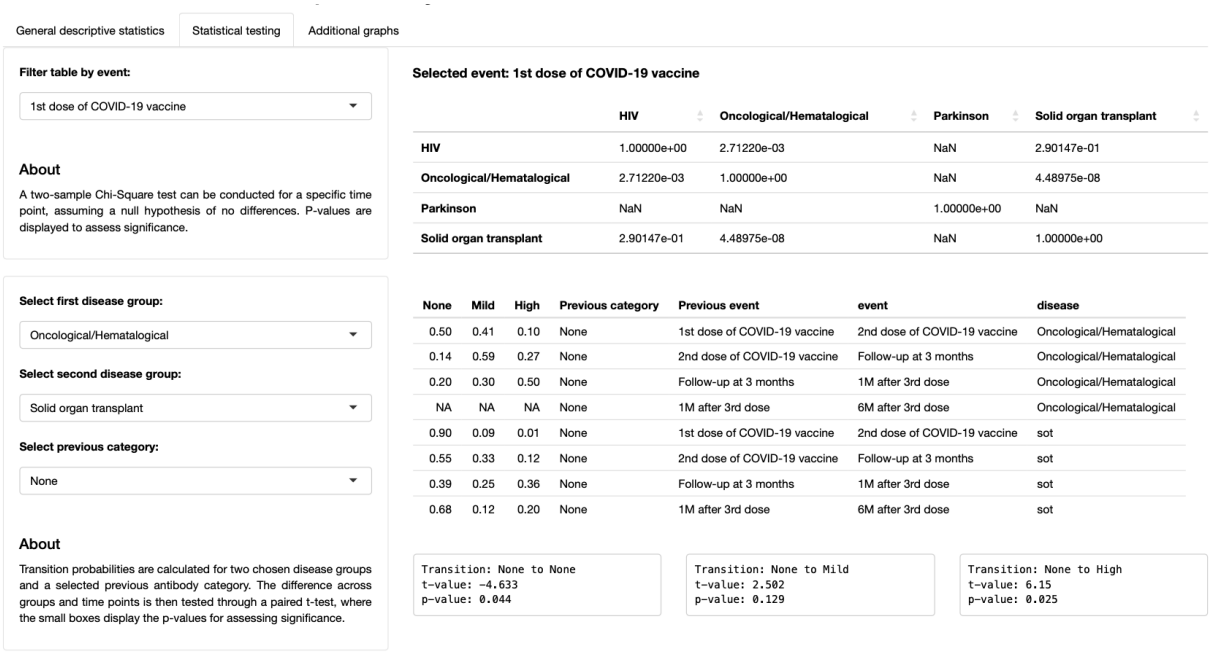

Figure 2: Overview of the statistical testing section within the R Shiny online dashboard across different patient groups, time points and previous immune system responses. Access to the app via: https://apps-carolina2107.shinyapps.io/shiny/ ; username: shiny-orchestra ; password: HFNSGHRI759261

When observing the frequency distributions for the different fragile groups and antibody categories, the results show heterogeneous patterns over time. However, a general trend emerges showing that antibody levels in all disease groups tend to rise over time.

For this specific analysis, the SOT group was broken down into sub-groups to provide a clearer picture of the antibody distributions across various types of transplant. In the same manner, data of patients with solid cancer and hematological malignancy were further divided.

Then, the HIV and Heart Transplant (from the SOT group) patients were used as illustrative examples, largely due to their similar sample sizes. HIV group's shows a rapid progression, most notably one month after the 3rd dose, marked by a sharp uptick in the high antibody category (Figure 3A). In contrast, the Heart Transplant group, despite an overall increase in antibody levels, progresses to the high category at a more gradual pace by that same juncture (Figure 3B).

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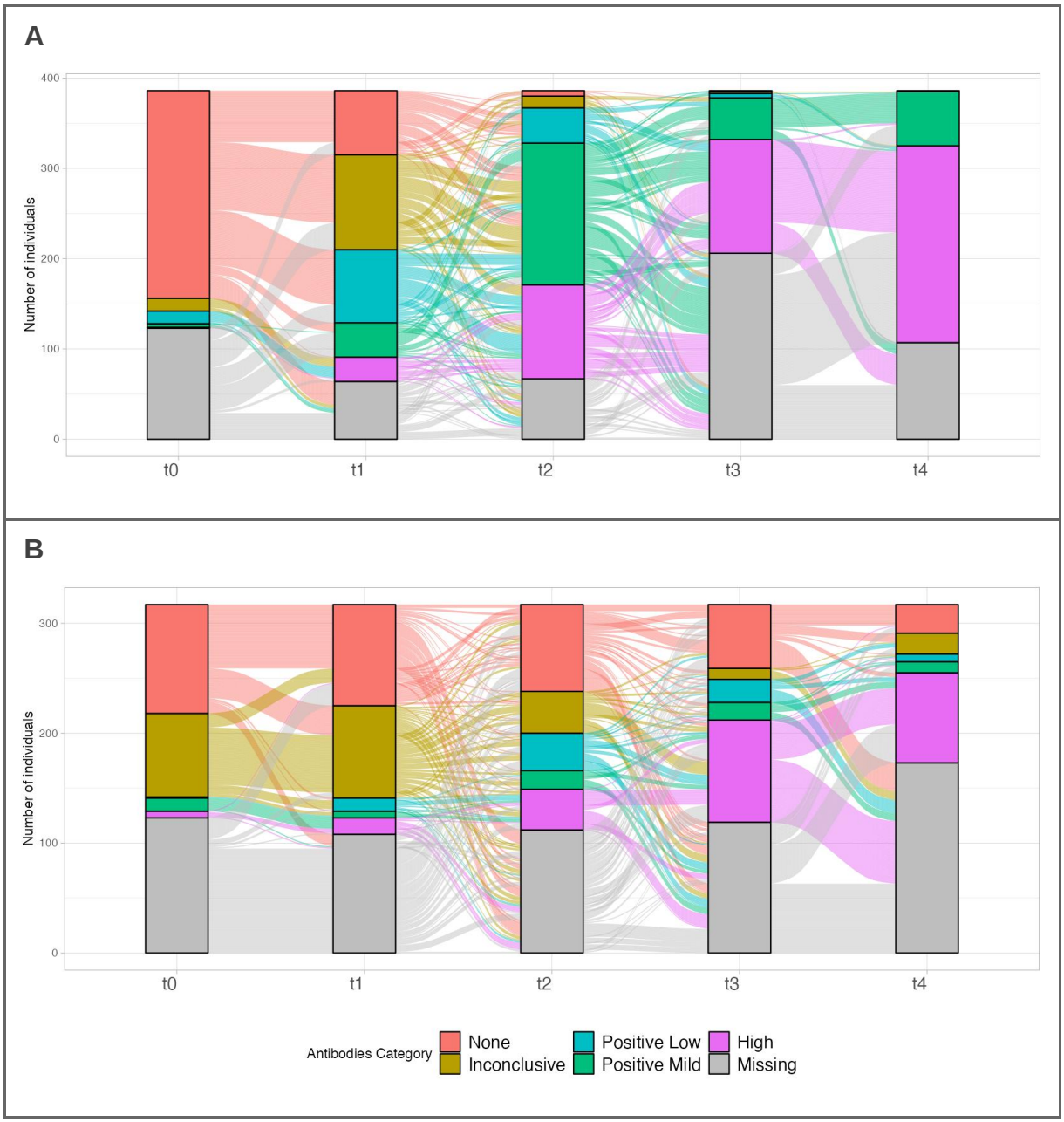

Figure 3: Alluvial plots illustrating the dynamic shifts in antibody response categories over time (t0-t4). **A:** HIV disease group. **B:** Heart Transplant disease group.

Upon examining the statistical tests, the Chi-Square test results provide evidence of potential differences in antibody response distributions among the fragile patient groups at specific time points, with a null hypothesis of no difference. The results indicate pronounced disparities in antibody response distribution between the patient cohorts. For instance, at early time points

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(Table 1A), all p-values are below the 1% significance threshold (< 0.01). This suggests strong evidence of distinct antibody distributions across all patient groups at this juncture, except for the Parkinson's disease group, which lacks data at this timepoint. These differences persist in later time frames (Table 1B).

	HIV	Oncological or Hematological	Parkinson	Solid Organ Transplant
A: time point t1				
HIV	1.00	<0.0001	N/A	<0.0001
Oncological or Hematological	<0.0001	1.00	N/A	<0.0001
Parkinson	N/A	N/A	1.00	N/A
Solid Organ Transplant	<0.0001	<0.0001	N/A	1.00
B: time point t4				
HIV	1.00	<0.001	0.000237	<0.0001
Oncological or Hematological	<0.0001	1.00	0.2575	<0.0001
Parkinson	0.000237	0.2575	1.00	N/A
Solid Organ Transplant	<0.0001	<0.0001	N/A	1.00

Table 1: P-values from the Chi-Square tests comparing the antibody distributions between groups, where N/A indicates no available data for the comparison.

Further analysis using paired t-tests assesses differences in transition probabilities, conditioned on the fragile population groups being compared and their prior antibody category measurements (e.g., non-reactive, mild, or high). The outcomes of these tests are diverse. However, a hands-on illustration is seen in the paired t-tests between the HIV disease group and the Solid Organ Transplant (SOT) disease group (Figure 4). From a broader perspective, while there are variations in transition probabilities between the HIV and SOT groups, the only statistically significant difference observed is for patients maintaining a non-reactive response without transitioning to any higher antibody state over time, reflected by a p-value of 0.013 (<

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0.05). This indicates that, for this specific transition, the two groups follow different patterns. For all other transitions, the trajectories are not statistically different over time.

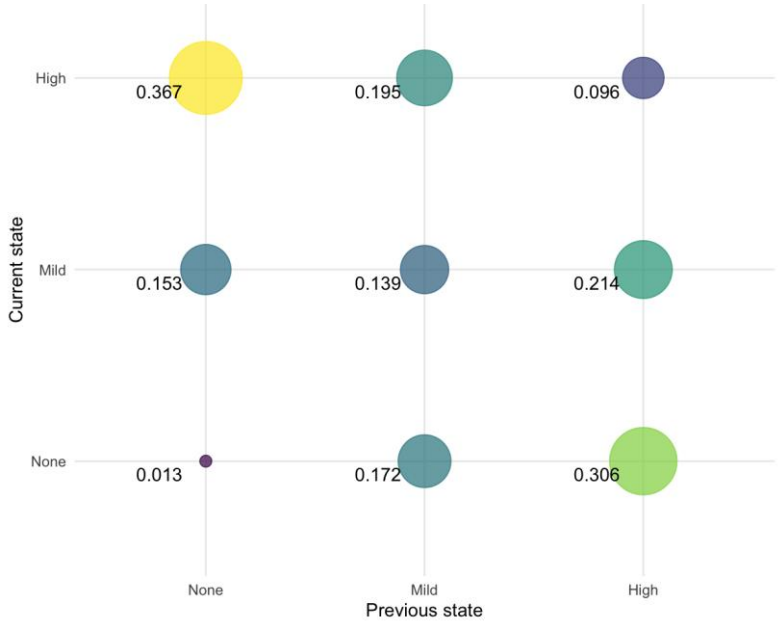

Figure 4: Visualization of pairwise p-values (bubbles) between antibody response categories of HIV and solid organ transplant disease group for all time points (t0-t4). Smaller bubbles indicate higher significance and evidence against the null hypothesis of no difference in transition probabilities across time for these two disease groups.

Diving into the transition probabilities at particular timepoints gives further insights into the evolution of the antibody response. These results vary across disease groups and timeframes, but focusing on the HIV and SOT groups provides clarity, especially in the context of the prior t-test findings. For these specific disease groups, there is evidence for a distinct difference in the non-reactive category at early time points t0 to t1 (Figure 5). Both groups show similar tendencies to transition to higher antibody levels if initially categorized as mild or high—however, those who began as non-reactive manifest different trajectories. While the HIV group leans more toward transitioning to higher antibody levels (Figure 5A), the SOT group remains essentially non-reactive (Figure 5B). This disparity is accentuated at later time points, where SOT patients continue to exhibit a higher likelihood of remaining non-reactive, compared to the HIV group which shows no such trend. Conclusively, every fragile disease group, when considered alongside initial antibody levels, displays distinct patterns in subsequent immune reactions over time.

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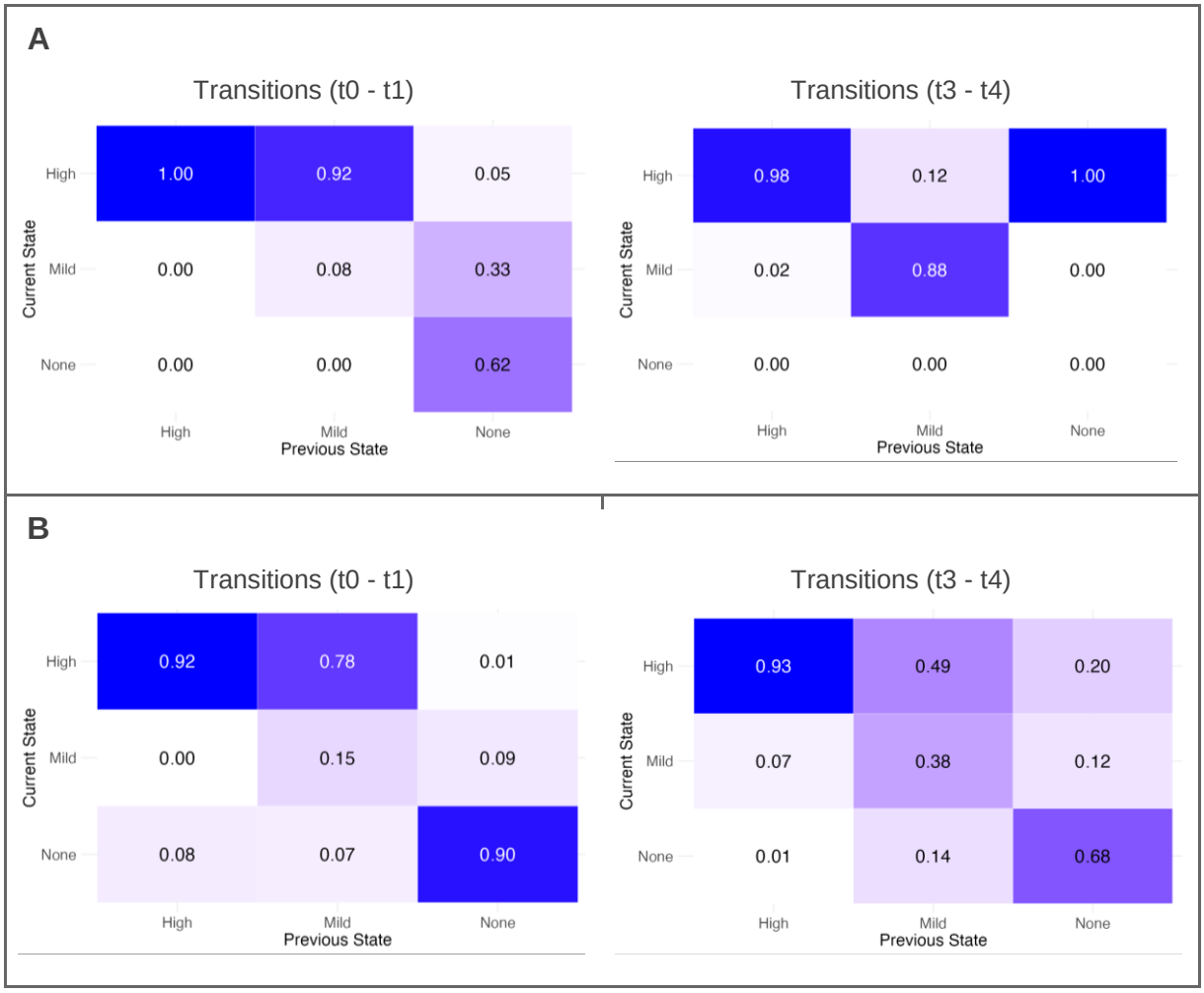

Figure 5: Heatmaps with transition probabilities across time points. The color intensity of each tile indicates the likelihood of transition from the previous state to the current state, with darker shades representing higher probabilities. **A**: HIV disease group. **B:** SOT group.

Discussion

For the first time, antibody response to SARS-CoV2 vaccine between several types of fragile patients receiving a complete vaccine course plus at least one booster dosage is provided. Our study confirms that among all fragile patients, some groups such as heart transplant recipients took longer in mounting a high-level antibody response (AbR) compared to other fragile patients, in particular those with HIV. In addition, SOT recipients showed a higher probability compared with all the other fragile patients to still have a null antibody response even after the booster dosage. As a consequence, the transition probability to a high AbR level at the last timepoints (1 and 6 months after booster dosage) was lower in SOT recipients than in other fragile patients.

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In an open-label, noninferiority randomized clinical trial 341 patients living with HIV and 71 SOT recipients were randomized to mRNA-1273 or BNT162b2 (8) The primary endpoint was antibody response to SARS-CoV-2 spike (S1) protein receptor binding domain (Elecsys Anti-SARS-CoV-2 immunoassay, Roche; cutoff ≥0.8 units/mL) 12 weeks after first vaccination (i.e., 8 weeks after second vaccination). The percentage of patients showing an immune response was 92.1% (95% confidence interval [CI]: 88.4-95.8; 186/202) for mRNA-1273 and 94.3% (95% CI: 91.2-97.4; 198/210) for BNT162b2 (difference: -2.2%; 95% CI: -7.1 to 2.7), fulfilling noninferiority of mRNA-1273. All PLWH had an antibody response (100.0%; 341/341), whereas for SOT recipients, only 60.6% (95% CI: 49.2-71.9; 43/71) had titers above the cutoff level.

In a monocentric prospective cohort study, conducted at Geneva University Hospitals, anti-SARS-CoV-2 spike protein antibody titers following two and three doses of mRNA vaccines in four groups of immunocompromised patients (cancer, n=232; hematopoietic stem cell transplant recipients, n=126; people living with HIV, n=131; and lung transplant recipients, n=39), and in healthy individuals (n=49) were assessed (9). After first vaccination dose, the highest anti-S antibody geometric mean titer (IU/mL) was observed in healthy individuals (2417 IU/mL [95% CI: 2327-2500]), the HIV group (2024 IU/mL [95% CI:1854-2209]) and patients with cancer (840 IU/mL [95% CI: 625-1129]), whereas patients in the HSCT and LT groups had weaker antibody responses (198 IU/mL [95% CI: 108-361] and 7.3 IU/mL [95% CI: 2.5-22]). The booster dose conferred a high antibody response after 1 month in both HIV (2500 IU/mL) and cancer patients (2386 IU/mL [95% CI: 2182-2500]), a moderate response in HSCT patients (521 IU/mL [95% CI: 306-885]) and a poor response in LT recipients (84 IU/mL [95% CI: 18-389]).

Strengths of our study include the heterogeneity and large size of the cohort longitudinally tested; the prospective assessment of antibody response according to a common protocol; the availability of early and late timepoints after vaccination onset. The main limitation is the missing of data related to late timepoints generally because of logistical reasons (i.e., patient difficulties in returning to referral center, center difficulties in shifting timepoints according to different vaccination schedules). Thus, we may assume that the data in the study are missing at random. The missing data is not associated with other observed factors in our study, and in particular to the actual unobserved antibody response. Its statistical implication is that the missing data will not introduce a systematic bias in the estimated results. The patterns of

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missingness, while potentially problematic for reducing our effective sample size, will not skew our results in a specific direction.

Our findings confirmed the heterogeneity of the humoral response after mRNA vaccines among different fragile patients. We deem these results could be useful to select priority groups for booster dosages and/or for recommendation regarding the assay of immunization status in order to implement adjunctive strategies.

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References

1. Kwon JH, Tenforde MW, Gaglani M, Talbot HK, Ginde AA, McNeal T, et al. mRNA Vaccine Effectiveness Against Coronavirus Disease 2019 Hospitalization Among Solid Organ Transplant Recipients. J Infect Dis. 2022 Sep 13;226(5):797–807.

2. Lee ARYB, Wong SY, Chai LYA, Lee SC, Lee MX, Muthiah MD, et al. Efficacy of covid-19 vaccines in immunocompromised patients: systematic review and meta-analysis. BMJ. 2022 Mar 2;e068632.

3. Sakuraba A, Luna A, Micic D. A Systematic Review and Meta-Analysis of Serologic Response following Coronavirus Disease 2019 (COVID-19) Vaccination in Solid Organ Transplant Recipients. Viruses. 2022 Aug 19;14(8):1822.

4. Giannella M, Righi E, Pascale R, Rinaldi M, Caroccia N, Gamberini C, et al. Evaluation of the Kinetics of Antibody Response to COVID-19 Vaccine in Solid Organ Transplant Recipients: The Prospective Multicenter ORCHESTRA Cohort. Microorganisms. 2022 May 12;10(5):1021.

5. Bonazzetti C, Tazza B, Gibertoni D, Pasquini Z, Caroccia N, Fanì F, et al. Relationship Between Immune Response to Severe Acute Respiratory Syndrome Coronavirus 2 Vaccines and Development of Breakthrough Infection in Solid Organ Transplant Recipients: The CONTRAST Cohort. Clin Infect Dis. 2023 May 24;76(10):1761–7.

6. Giannella M, Huth M, Righi E, Hasenauer J, Marconi L, Konnova A, et al. Using machine learning to predict antibody response to SARS-CoV-2 vaccination in solid organ transplant recipients: the multicentre ORCHESTRA cohort. Clin Microbiol Infect. 2023 Aug;29(8):1084.e1-1084.e7.

7. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform. 2019 Jul;95:103208.

8. Speich B, Chammartin F, Abela IA, Amico P, Stoeckle MP, Eichenberger AL et al. Antibody Response in Immunocompromised Patients After the Administration of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Vaccine BNT162b2 or mRNA-1273: A Randomized Controlled Trial. Clin Infect Dis 2022 Aug 24;75(1):e585-e593.

9. Bordry B, Mamez AC, Fedeli C, Cantero C, Jaksic C, Ustero Alonso P et al. SARS-CoV-2 m-RNA Vaccine Response in Immunocompromised Patients: A Monocentric Study Comparing Cancer, People Living with HIV, Hematopoietic Stem Cell Transplant Patients and Lung Transplant Recipients. Vaccines (Basel) 2023 Jul 26;11(8):1284.

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WP4_Deliverable 4.4

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PEDIATRIC POPULATION

(PENTA)

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1. Executive summary

This deliverable is part of Work package 4. One of the main objectives of this WP is to evaluate and characterize the immune response to SARS-CoV-2 vaccines in fragile populations, including children and pregnant women.
Among the children cohorts involved in Orchestra, Case cohort and EPICO decided to develop a specific prospective protocol in order to contribute to this research question. The purpose of this document is to summarize the work and results achieved so far by these two paediatric cohorts.

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2. Pediatric populations

2.2 Case cohort

Country	Italy - Padova
Type of cohort	Household
Study design and methods	This is a multicenter, prospective, cohort study on children aged 5-11 years old, attending the COVID-19 Family Cluster Follow-up Clinic (CovFC) set up at the Department of Women's and Children's Health of the University Hospital of Padua. The study population includes: a. Infected and vaccinated healthy children aged 5-11 years b. Un-infected and vaccinated healthy children aged 5-11 years c. Infected and vaccinated immunocompromised patients aged 5-11 years (e.g., solid organ transplant recipients on anti-rejection regimen and patients with immune-inflammatory diseases on immunomodulating therapy) d. Un-infected and vaccinated immunocompromised patients aged 5-11 years e. Children aged 5-11 years with a complicated SARS-CoV-2 infection (multisystem inflammatory syndrome in children – MIS-C).
Study Objectives	*Primary objectives:* 1. To describe the characteristics and long-term kinetics of the humoral and cellular immune response induced by administration of the BNT162b2 vaccine in children aged 5 to 11 years with previous SARS-CoV-2 infection compared to naïve-vaccinated 5-11-years-old individuals 2. To compare the immune response after COVID-19 vaccination between healthy 5–11-year-olds and Immunocompromised peers.
Methods:	Patients were enrolled in the study before obtaining the mRNA vaccination if attending the following criteria: a) being between the ages of 5 and 11 years at the time of vaccination and b) one of the following: • receiving a 2-doses primary series of the COVID-19 mRNA BNT162b2 vaccine (Pfizer-BioNTechComirnaty) • receiving at least one dose of the BNT162b2 vaccine within 11 months after a previous laboratory-confirmed COVID-19. At enrolment, a pediatrician collected data on demographic parameters, pertinent past medical history, including chronic ongoing therapies, and previous SARS-CoV-2 infection. Moreover, children underwent clinical evaluation and a blood sample collection for characterization of the immune profile to SARS-CoV-2 before vaccination.

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	Then children received one or two 10-µg doses of the BNT162b2 vaccine with an interval between doses of 21 days as was recommended by the Food and Drug Administration for this age group when the study was conducted. After vaccination, children were followed up for clinical and longitudinal immunological evaluation. Data on new contacts with confirmed COVID-19 cases or with probable COVID-19 cases, as well as any breakthrough infection were collected at each visit. In case of new contact or infection with SARS-CoV-2 the follow-up was interrupted. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-containing tube at 1 and 6 months after the 1- or 2-doses primary series of the BNT162b2 vaccine. Plasma, serum, and cells were separate by Ficoll–Paque gradient (Pharmacia, Uppsala, Sweden). Sera and Plasma were collected, centrifuged, and appropriately stored at −80°C until use. Cells were appropriately stored in liquid nitrogen. Bloods was tested for anti-SARS-CoV-2 S-RBD IgG antibodies, PRNT50 neutralizing antibodies, and B and T immunophenotype. Information on patients' clinical characteristics and laboratory findings collected at enrollment and during follow-up were anonymized and entered into a web-based database using the REDCap® platform (Vanderbilt University, Tennessee) hosted in the server of the University of Padova. <u>*Serological assays*</u> Quantification of anti-SARS-CoV-2 S-RBD IgG Ab was performed through commercially available chemiluminescent assays (CLIA) (Snibe Diagnostics, New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China). (ref. 130219017M). This method, previously validated elsewhere, quantitatively determine the IgG antibodies to RBD portion of SARS-CoV-2 spike protein. All analyses were conducted on MAGLUMI™ 2000 Plus (Snibe Diagnostics), with results expressed in kilo Binding Antibody Unit (kBAU/L), in accordance to WHO International Standard for anti-SARS-CoV-2 immunoglobulin. Samples recording titers >4.33 kBAU/L were considered positive. A high-throughput method for Plaque Reduction Neutralization Test (PRNT) was used to quantify neutralizing antibodies (NAbs) in serum samples against the Parental variants of concern. The neutralization titer was defined as the reciprocal of the highest dilution resulting in a reduction of the control plaque count >50% ($PRNT_{50}$). Samples recording titers equal to or above 1:10 were considered positive. <u>*Immune assays*</u> The immune profiles of activation, senescence, exhaustion, and regulatory cells were analyzed by flow cytometry.

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	Cells were thawed, washed, stained for 20 min in the dark with the Live/Dead Fixable Near-IR Dead Cell Stain Kit (Life Technologies, Carlsbad, California, USA) and the following labelled monoclonal antibodies: anti-CD3 [fluorescein isothiocyanate (FITC)], anti-CD4 [peridinin chlorophyll protein (PerCP)], anti-CD38 [phycoerythrin (PE)], anti-HLA-DR [allophycocyanin (APC)], anti-CD27 [PE], anti-CD45RA [APC], anti-CCR7 [PE-Cy7], anti-CD28 [BV421], anti-CD57 [PE-CF594], anti-CD279 (programmed cell death 1, PD-1) [PE-Cy7], anti-TIGIT [BV605], anti-CD21 [BV421], anti-CD27 [PE-Cy7], anti-IgD [PE] (Becton-Dickinson-BD, San Diego, California, USA); anti-CD8 [VioGreen], anti-CD19 [VioBright515], anti-CD10 [APC] (MiltenyiBiotec, Auburn, California USA). Cells were then washed and resuspended in PBS supplemented with 1% paraformaldehyde. T-regs were determined using anti-CD4 [BB515], anti-CD25 [BV421], anti-CD127 [PE-CF594] (Becton-Dickinson, San Diego, CA, USA) and combined membrane and intracytoplasmic staining for anti-FoxP3 [AlexaFluor 647] using a transcription factor buffer set according to the manufacturer's protocol (Becton-Dickinson). All samples were analysed using LSRII Flow cytometer (Becton-Dickinson). A total of 50 000 events were collected in the lymphocyte gate using morphological parameters (forward and side-scatter). Data were processed with FACSDiva Software (Becton-Dickinson) and analysed using Kaluza Analyzing Software v.1.2 (Beckman Coulter, Brea, CA, USA). *Case identification and definitions* Study participants were divided into 3 groups according to their past medical history. *Group 1* of "healthy" children; *group 2* of children with underlying diseases which required immunomodulatory and/or immunosuppressant (anti-rejection) therapy; *group 3* of children who developed a multisystemic inflammatory syndrome (MIS-C) after COVID-19. Children who got SARS-CoV-2 infection before vaccination were considered *COVID-19 cases*; on the other hand, children with no history of previous COVID-19 and without serological evidence of SARS-CoV-2 infection before vaccination (negative anti-S-RBD IgG and negative NAbs) were defined as *non-COVID-19 cases*. *COVID-19 cases* enrolled in the study were included in the statistical analysis if a defined *baseline date* was present. For each *COVID-19 case*, a *baseline date* was defined as follows: 1) for symptomatic cases: the first date between the onset of symptoms or the date of first positive SARS-CoV-2 molecular assay; 2) for asymptomatic cases: the date of the first positive molecular assay or, in those with only serologically confirmed COVID-19 and with negative/undetermined nasal-pharyngeal swab (NPs), by the family outbreak temporal sequence, coinciding with the date of symptoms onset in the family cluster.

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	The severity of COVID-19 was scored as mild, moderate, severe, critical, or MIS-C following the WHO classification. *Statistical analyses* Descriptive statistics, Chi-squared test, Fisher's exact test, and Student's t-test were used for either categorical or continuous covariates appropriately. The Abs titers response was assessed by comparing the median and the interquartile range (IQR) of anti-SARS-CoV-2 S-RBD IgG values and the geometric mean titer (GMT) and the 95% confidence interval (95%CI) in the overall dataset, including both independent and subject-paired samples, and stratified by age classes (age 5-7 years and 8-11 years), and by the time between vaccination and baseline of previous infection. The Kruskal-Wallis test was performed, accordingly. Associations between antibody titers, baseline intervals and age, were assessed with linear regression models.
Summary of results	From December 2021 to February 2023, we prospectively evaluated 81 Italian children of 5-11 years old (35 [43%] females). Of these, 21 developed a laboratory-confirmed COVID-19 after vaccination and therefore were excluded from the analysis. Among 60 patients that were included in the study, 46 had a previous history of COVID-19 (COVID-19 cases) and 14 had no analytical evidence of a previous SARS-CoV-2 infection (non-COVID-19 cases). Overall, 40 children were healthy [HC], 8 children had an underlying immune-inflammatory chronic diseases requiring an immunomodulatory treatment [IM], 4 children were on anti-rejected regimen for a solid organ transplant [SOT], and 8 children had previous MIS-C [MIS-C]. Overall, we observed a higher humoral response (in terms of anti-S-RBD IgG and NAbs) after the BNT162b2 vaccine in COVID-19 cases compared to non-COVID-19 cases, regardless an immunocompromised condition or a previous MIS-C at both one ($p<0.0001$) and 6 months ($p<0.0001$) follow up. Stratifying patients according to comorbidities, we noticed that while HC, IM, and MIS-C recovered a satisfactory anti-S-RBD IgG and NAbs titers, SOT had a reduced humoral response at both time-points follow-up. All patients developed a significant decrease in antibodies titers between 1 and 6 months after vaccination, regardless a previous history of COVID-19 as well as an underlying condition or MIS-C. However, all COVID-19 cases recorded higher anti-S-RBD IgG and NAbs titers at 6 months after vaccination compared to pre-vaccination. Immunological parameters were compared between COVID-19 and non-COVID-19 cases at 1 and 6 months after vaccination, and also compared by stratifying according to their health status in HC, IM, SOT, and MIS-C. First of all, no difference in the frequencies of T (CD4 and CD8) and B activated and

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	senescent cells between COVID-19 and non-COVID-19 patients at both timepoints; while the frequencies of T- and B-regs were significantly higher in COVID-19 cases than non-COVID-19 both at 1 (T-reg, p=0.023; B-reg, p=0.140) and 6 months (T-reg, p=0.020; B-reg, p=0.034) after vaccination. This trend could be appreciated among HC, IC, SOT, and MIS-C children; indeed, despite previous COVID-19 infection, similar percentages of activation and senescent T and B cells were found among the groups, but for each subgroup, regulatory T and B cells were higher in COVID-19. All these findings will be presented in a final manuscript.

2.2 Epico

Country	Spain
Type of cohort	Hospital
Study design	This is a multicenter, observational cohort study that took place in several sites in Spain. 82 children aged 5-11 years which received a course of mRNA SARS-CoV2 vaccine between December 2021 and May 2022 were followed-up and divided in two groups (41 in each group): ● Healthy children not immunocompromised ● Immunocompromised children (e.g., children who are organ transplant, receiving dialysis, have had chemotherapy or radiotherapy in the previous 6 months, with immunodeficiency). The vaccinations were scheduled as below: Healthy children without SARS-CoV-2 infection received two vaccine doses with an 8-week interval. If the child became infected with SARS-CoV-2 after the first dose and before the second, the second dose was administered 4 weeks after SARS-CoV-2 infection diagnosis, after full clinical recovery (always maintaining ≥ 8-week interval). Children with SARS-CoV-2 infection before the first dose received a single dose 4 weeks after SARS-CoV-2 infection diagnosis, after full clinical recovery. Immunocompromised children received three doses with 8 weeks between the 1st and 2nd doses and a ≥28 days interval between the 2nd and 3rd dose. If the child acquired SARS-CoV-2 infection, the next dose has been done after 4 weeks; all children have received 3 doses. Patients enrolled in the study had blood samples taken at baseline (before vaccination), after the first and third dose, and 1 and 6 months after completion of the vaccination cycle. These samples were tested for anti-SARS-CoV-2 S-RBD IgG antibodies and neutralizing antibodies.
Study objectives	1. To characterize the immune response to mRNA SARS-CoV-2 vaccines in vaccinated children 5 to 11 years of age according to immune status (immunosuppressed vs immunocompetent). 2. To describe SARS-CoV-2 infections previous to vaccination, during vaccination and after complete vaccination (breakthrough infections)

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	3. To evaluate the impact of previousSARS-CoV-2 infection on the immune response to mRNA SARS-CoV-2 vaccines in vaccinated children aged 5 to 11 years according to immune status 4. To describe adverse events (AE) and serious adverse events (SAE) after vaccination doses *5.* If new vaccines are licensed, to compare immunogenicity, and SAEs of different vaccines in the same groups.
Methods:	The study involved 3 Spanish hospitals with 33 patients enrolled in total until now (6 immunosuppressed and 27 controls). A total number of 156 samples were collected: 12 samples at baseline (before vaccination), 96 samples at 1 month and 45 at 6 months after vaccination. Children from 5 to 11 years vaccinated with Pfizer/BioNTech BNT162B2 were recruited in December 2021. Sera were obtained at 1 (+1m) and 6 months (+6m) after full vaccination: 2 doses for healthy participants (HP), 3 for immunosuppressed participants (ISP). Neutralizing activity for ancestral, beta (B.1.351), delta (B.1.617.2) and omicron variants (BA2.12.1, BA.2, BA.2+L452M, BA.2+L452R, BA.3, BA.4, BA.5) was analyzed.
Summary of results:	1. Neutralizing activity after BNT162B2 was high for ancestral, beta and delta at +1m but low at +6m. No differences between HP and a limited number (n=6) of ISP children were observed. Response to omicron was low at all time points. 2. 18 children were followed for 12 months after vaccination. 5/12 children had either PCR-confirmed COVID-19 or increased anti-N titers between vaccination and 6-months follow-up. Median IFN-γ after CD4 stimulation (n=18) was 0.72 (0.24-2.04) IU/mL. CD4 response positively correlated with simultaneous serological IgG responses against various SARS-CoV-2 antigens. We propose anti-S antibodies as a proxy of cellular immunity. 3. 2/12 children reported COVID (PCR+) up to 6 months after vaccination. In both cases, CD4 response was <0.2 IU/mL. No children with >0.2 IU/mL reported COVID-19 in the 6 months after testing. A CD4 response >0.2 IU/mL may confer protection for at least 6 months. Only 2 breakthrough infections were detected after vaccination. 2/12 (16.67%) children reported COVID (PCR+) up to 6 months after vaccination. In both cases, CD4 response was < 0.2 IU/mL. No children with >0.2 IU/mL reported COVID-19 in the 6 months after testing. 4. Prior infection did not show significant differences in the immune response to the vaccine. 5. Adverse effects of the SARS-CoV-2 vaccine in children were frequent, but local and of short duration. COVID-19 infection or immunosuppression did not influence the frequency or intensity of adverse effects. 6. No new vaccines were licensed for 5 to 11 years. 7. Mostly heathy participants were included, only 6 IS participants reached timepoints, due to irregular 3rd dose of vaccination of immunosuppressed patients, as well as withdrawal of consent and rejection to extra blood extractions. As time progressed, and Omicron's low severity was demonstrated, more IS patients withdrew.

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Test	Total	Healthy	Immunosuppressed	p-value	N
	n=26	n=20	n=6		
Ac IgG anti-S (CLIA) 6 months	1864 [1001;3175]	1864 [1001;3175]	-	-	12
Ac IgG anti-S (CLIA) 1 month	4676 [3037;9559]	4913 [2644;8239]	4098 [4028;11209]	0.885	26
Ac neutralizing 6 months	12.1 [7.27;28.9]	12.1 [7.27;28.9]	-	-	12
Ac neutralizing 1 month	49.3 [12.5;68.4]	55.6 [5.42;68.4]	42.4 [37.3;63.0]	0.885	26
Quantiferon (n, %)					
Positive	16 (88.9%)	14 (87.5%)	2 (100%)	-	18
Negative	2 (11.1%)	2 (12.5%)	0 (0.0%)		
Response CD4	0.72 [0.28;2.32]	0.65 [0.24;1.90]	2.20 [1.54;2.87]	0.567	18

Feature	Total	Healthy	Immunosuppressed	p-value
	n=26	n=20	n=6	
Age (Average, DE)	9.27 (1.79)		8.85 (1.69)	
		9.39 (1.84)		0.511
Sex (n, %)				0.215
Female	15 (57.7%)	13 (65.0%)	2 (33.3%)	
Biologic	3 (11.54%)	0	3 (50.0%)	
Quimiotherapy	3 (11.54%)	0	3(50.0%)	

Humoral response	CD4 response		
	Cor	β (95% CI)	p-value
IgG anti-S (CLIA) 6 months	0.7672	0.0006 [0.0002;0.0010]	0.0059
Ab neutralizing 6 months	0.6084	0.0635 [0.0010;0.1259]	0.0470

Adverse events	Total	Healthy	Immunosuppressed	p-value
	n=26	n=20	n=6	
Adverse events 1 dosis (n, %)	13 (50.0%)	12 (60.0%)	1 (16.7%)	0.088
Adverse events 2 dosis (n, %)	9 (34.6%)	9 (45.0%)	0 (0.00%)	-
Type Adverse events 1 dosis (n, %)				
Fatigue	1 (3.8%)	0 (0.00%)	1 (16.7%)	-
Local pain	12 (46.2%)	12 (60.0%)	0 (0.00%)	
None	13 (50.0%)	8 (40.0%)	5 (83.3%)	
Type Adverse events 2 dosis (n, %)				
Fatigue	1 (3.8%)	1 (5.0%)	0 (0.0%)	-
Local pain	6 (23.1%)	6 (30.0%)	0 (0.0%)	
Fever	1 (3.8%)	1 (5.0%)	0 (0.0%)	
Headache	1 (3.8%)	1 (5.0%)	0 (0.0%)	
None	17 (65.4%)	11 (55.0%)	6 (10%)	
Breakthrough infection				
	2 (7.6%)	2 (10.0%)	0 (0.0%)	-

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