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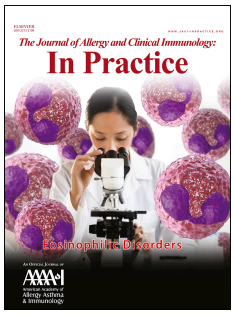

COVID-19 severity, cardiological outcome and immunogenicity of mRNA vaccine in adult patients with 22q11.2DS

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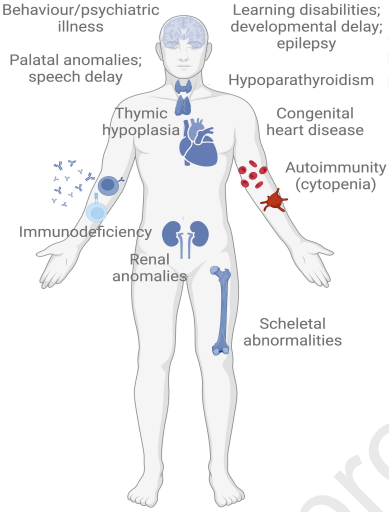
SARS-CoV-2 infected

Disease severity	Asymptomatic/Mild: 95% Died: 5%
Symptoms	Comparable to those experienced by the general population
Cardiological outcome	Congenital heart disease: no worsening; No new/late cardiological manifestation
Risk factor	Lymphocytes count <1500; Low CD4+ count; Moderate/severe intellectual disability
Treatment	One out three receiving prophylaxis with monoclonals or antiviral

22q11.2 deletion syndrome



mRNA BNT162b2 vaccinated

Robust respose after 3rd dose	anti S1-IgG
Weaker response than HD after 3rd dose	anti S1-IgA
Weaker response than HD after 3rd dose	Spike specific MBCs
Detectable, but lower in those with lymphopenia and low CD4+ count	Antigen specific T cells
Only in 50% of DS patients	VOC specific T cells

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COVID-19 severity, cardiological outcome and immunogenicity of mRNA vaccine in adult patients with 22q11.2DS

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Abstract

Background. The contemporaneous presence of immune-defects and heart diseases in patients with 22q11.2 deletion syndrome might represent risk factors for severe COVID-19.

Objective. To analyze SARS-CoV-2 outcome in 22q11.2DS patients and immunogenicity of different doses of mRNA SARS-CoV-2 vaccine.

Methods. Longitudinal observational study on SARS-Cov-2 outcome in 60 adults with 22q11.2DS (March 2020-June 2022). Anti-Spike, and anti-receptor binding domain antibody responses, generation of Spike-specific memory B-cells and Spike-specific T-cells at different time points before and after the mRNA BNT162b2 vaccination were evaluated in sixteen 22q11.2DS patients.

Results. We recorded a 95% rate of vaccination, with almost all patients being immunized with the booster dose. Twenty-one patients had SARS-CoV-2 infection. Three patients were infected before vaccine availability, six after receiving two doses of vaccine and twelve after the booster dose. SARS-CoV-2- infection had a mild course, except one unvaccinated patient with several comorbidities who died from acute respiratory distress syndrome (fatality-rate: 5%). Infected patients had more frequently moderate/severe intellectual disability, lymphopenia and lower CD4+ count. Despite major congenital heart diseases, COVID-19 did not impact cardiological conditions. The BNT162b2 vaccine induced S1-IgG responses, low serum S1-IgA, and slightly impaired specific memory B-response. Specific T-cell responses observed were related to lymphocytes and CD4+ T cell counts.

Conclusion. SARS-CoV-2 infection had a mild course in most patients with 22q11.2DS, even in patients with major cardiovascular diseases. Immunization induced Spike-specific IgG responses and generated specific memory B and T cells. The weaker memory responses in patients with lymphopenia suggested the need for additional doses.

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Highlights box

- **What is already known about this topic?** At present, data on the course of SARS-CoV-2 infection in 22q11.2DS patients are scarce and limited mainly to pediatric case reports. Data on immunological response to immunization are lacking.
- **What does this article add to our knowledge?** SARS-CoV-2 infection in 22q11.2DS had a mild course in most patients, even in those with major cardiovascular diseases. Lymphopenia represents a risk factor for becoming infected. The mRNA BNT162b2 vaccine induced Spike-specific IgG responses and generated specific memory B and T cells.
- **How does this study impact current management guidelines?** The weaker memory responses in patients with lymphopenia suggested the need for periodic reassessment of serology to identify patients needing additional recall dose administration. Fatal course in one unvaccinated person highlight the importance of immunzation to protect this population from severe COVID-19

Key words: SARS-CoV-2 infection, COVID-19, 22q11.2 deletion syndrome, cardiovascular disease, mRNA BNT162b2 vaccine, Spike antibody response, Specific memory B cells, Specific T cells, Lymphopenia, CD4 T cells.

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Conflicts of interest: The authors declare they have not any conflict of interest

List of abbreviations

22q11.2DS 22q11.2 deletion syndrome

BMI body mass index

CBC complete blood count

CHD Congenital Heart Diseases

FISH fluorescent in-situ hybridization

HD healthy donors

IAA interrupted aortic arch

IEIs Inborn Errors of Immunity

Ig immunoglobulin

MBC Memory B cell

NPS nasopharyngeal swab

NR nonresponders

PA+VSD pulmonary atresia and ventricular septal defect

RBD Receptor Binding Domain (RBD) specific B-cells

R Responders

SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2

ToF Tetralogy of Fallot

TA truncus arteriosus

VOC Variant of Concern

VSD isolated ventricular septal defects

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Introduction.

Despite being prioritized in vaccination campaigns, patients with Inborn Errors of Immunity (IEIs) became frequently infected with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), showing higher inpatient mortality at a younger age than the general population (1-4). Studies have been run to assess the efficacy of immunization strategies in IEIs (5-8). However, data on COVID-19 and immunological correlates of SARS-CoV-2 immunization in people with 22q11.2 deletion syndrome (22q11.2DS) are sporadic and refer primarily to pediatric patients (9-14) or self-completing surveys (15). Individuals with 22q11.2DS can present with a wide range of features, including immune deficiency, congenital heart diseases (CHDs), palatal abnormalities, learning difficulties, and neuropsychiatric disorders (16-18). Immunological defects are variable, ranging from the absence of the thymus with a SCID-like phenotype to less severe impairment with T-lymphocytopenia and autoimmunity (19-21). In adults, homoeostatic expansion and reconstitution of the T-cell compartment have been described, with the early conversion of naive to memory T-cells, shorter telomeres, and lower T-cell recombination excision circles, possibly leading to perturbation in T-cell function (22,23). In addition, hypogammaglobulinemia and abnormal B-cell function might contribute to infection recurrence (24,25) and reduced immunogenicity of vaccines (26-28). The presence of immune-defects and heart diseases (29), two conditions implicated in severe COVID-19, puts this population at high risk in the pandemic, such as adults and children with previous or preexisting cardiovascular conditions had an increased risk for severe COVID-19, such as adults and children with previous or preexisting cardiovascular conditions had an increased risk for severe COVID-19 (30,31). In addition, COVID-19 has been associated with developing several new cardiovascular pathologies (32). In the

22q11.2DS population, cardiac consequences of COVID-19 remain largely unknown, both in patients with and without CHD. In this study, we assessed the clinical course of SARS-CoV-2 infection by a longitudinal study of a monocentric cohort of 60 22q11.2DS adults, aiming to analyze if 22q11.2DS-associated conditions might affect the COVID-19 severity (and vice versa). As secondary objectives, we analyzed the antibody- and B/T-specific responses after two and three doses of the BNT162b2 mRNA-based SARS-CoV-2 vaccine.

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Methods

Study on COVID-19 infection. The observational-longitudinal study was carried out on 60 adults with 22q11.2DS followed up at Policlinico Umberto I, Sapienza University of Rome, between the 1st of March 2020 and the 30th of June 2022. All patients harbored a 22q11.2 microdeletion, verified through multicolor fluorescent in-situ hybridization (FISH) or by CGH array at the time of diagnosis.

Soon after the pandemic began, we informed all patients about the pandemic risk, prevention measures, and the need to contact the hospital in case of SARS-CoV-2 infection. Patients were tested for SARS-CoV-2 by RT-PCR on the nasopharyngeal swab (NPS) every time they attended a hospital site, in case of positive household contacts irrespective of symptoms, and upon onset of symptoms possibly related to COVID-19. In SARS-CoV-2 positive patients, we evaluated the duration of the viral shedding by recording the dates of the first positive and first negative NPS, COVID-19 severity (scored according to WHO stage (33), hospitalization, vaccination status, and SARS-CoV-2 specific treatments and cardiological outcome. Cardiological outcome was evaluated by chart reviews and self-reports during acute infection for out-patients, by medical files for hospitalized patients, and, after recovery, by transthoracic Doppler echocardiography and EKG at rest. For both infected and noninfected patients, we collected clinical characteristics, including neuropsychiatric, cardiovascular, and immunological diseases. Cardiovascular conditions included major CHD such as tetralogy of Fallot (ToF), pulmonary atresia and ventricular septal defect (PA+VSD), truncus arteriosus (TA), interrupted aortic arch (IAA), and isolated ventricular septal defects (VSD). In addition, we evaluated the presence of acquired conditions including arrhythmias, cardiac function impairment, previous heart surgery, and other. We also retrieved known risk factors for severe COVID-19 courses, such as overweight,

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obesity, hypertension, diabetes mellitus, intellectual and developmental disabilities, mental health disorders, smoking, use of corticosteroids or other immunosuppressive medications (34). Overweight and obesity were defined as having body mass index (BMI) 24.9-29.9 and >30, respectively. Immunological lab data included complete blood count (CBC) and immunoglobulin (Ig) serum levels.

Prospective study on immunological response to immunization. In six 22q11.2DS adults naive to SARS-CoV-2 infection, we carried out a pilot study on immunological response to BNT162b2 immunization after two vaccine doses. Then, we extended the study to sixteen 22q11.2DS adults naive to SARS-CoV-2 infection who received three doses of the BNT162b2 vaccine. The vaccine was administered in two doses, 21 days apart; the third dose was administered six months after completing the full immunization schedule. We obtained blood samples for serological and cellular immunity assessment before the first dose (T0), one week after the second dose (post 2D), six months after the second dose on the day of the third dose administration (pre 3D), and one week after the third dose (post 3D). Twenty-four age-matched healthy donors (HD) were included as controls. Eligible patients informed about the study and subscribed to informed consent for vaccination and the immunological study. The Ethical Committee of the Sapienza University of Rome approved the study (Prot. 0521/2020, the 13th of July 2020). The study was performed following the Good Clinical Practice guidelines, the International Conference on Harmonization guidelines, and the most recent version of the Declaration of Helsinki.

ELISA for Specific IgG and IgA detection. A semi-quantitative in vitro determination of anti-SARS-CoV-2 IgG and IgA was performed on serum samples using the Anti-

SARS-CoV-2 Spike ELISA (EUROIMMUN), according to the manufacturer's instructions and previously reported (5). Results are reported as the ratio between the OD sample and the OD calibrator. The ratio interpretation was as follows: < 0.8 = negative, ≥ 0.8 to < 1.1 = borderline, ≥ 1.1 = positive.

Cell Isolation and Cryopreservation. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll Paque™ Plus 206 (Amersham PharmaciaBiotech) density-gradient centrifugation and immediately frozen and stored in liquid nitrogen until use. The freezing medium contained 90% Fetal Bovine Serum (FBS) and 10% DMSO.

Detection of Antigen-Specific B-Cells. Recombinant biotinylated SARS-CoV-2 Spike (S1+S2; aa16-1211, R&D systems) was individually multimerized with streptavidin BUV395 (BD Bioscience) and streptavidin PE (BD Bioscience) at 25:1 ratio and 20:1 ratio, respectively at 4 °C for 1 h. Biotinylated RBD (kindly provided by Takis) was mixed with streptavidin-FITC (BD Bioscience) at 2.5:1 ratio. Non-specific streptavidin-binding B-cells were gated out with streptavidin PE-Cy7 (BD Bioscience). 5 × 10^6 previously frozen PBMC samples were stained with a 100 ng Spike *per* probe (total 200 ng), 27.5 ng of RBD, and 20 ng of streptavidin-PE-Cy7 at 4 °C for 30 min. After washing the cells, surface staining was performed in a brillant buffer at 4 °C for 30 min. Memory B cells (MBCs) were defined as CD19+CD24+CD27+ (Repository Figure E1, Gating strategy). MBCs specific for SARS-CoV-2 were distinguished by their ability to bind biotin-labeled recombinant Spike into S+ (PE single positive) or S++ (PE-BUV395 double positive). Among Spike specific MBCs we were able to identify RBD specific MBCs. Stained PBMC samples were acquired by FACS LSRFortessa (BD Bioscience). At least 4 × 10^6 cells were acquired and analyzed using FlowJo10.7.1

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(BD Bioscience). Phenotype analysis of antigen-specific B-cells was performed only when at least 10 cells were detected in the respective antigen-specific gate.

Detection of SARS-CoV-2-Specific T cell response. The frequency of Spike-specific T cells before and after vaccination was assessed by standard IFNγ ELISpot assay, as previously described (35,36). PBMC were thawed and rested overnight at 37°C in R10 medium [RPMI 1640 (Sigma Aldrich) supplemented with 10% heat-inactivated highly defined fetal bovine serum (FBS-HyClone), 2 mmol/L L-glutamine, 10 mmol/L HEPES buffer (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid, Sigma Aldrich), 100 U/ml penicillin, and 100 µg/mL streptomycin (Gibco)]. PBMC were plated at 3×10^5 cells/well in ELISpot plates (Human IFN-γ ELISpot plus kit; Mabtech) and stimulated for 18-20 hours at 37 ◦C (5% CO2) with a pool of peptides (Miltenyi Biotec) spanning the whole spike protein of the wild type SARS-CoV-2, or with a pool of peptides spanning the mutated portion of the Omicron Spike protein and, as a control, with a pool of peptides spanning the same region of the wild type Spike protein. A superantigen (SEB) was used as a positive control. At the end of incubation, the ELISpot assay was developed according to the manufacturer's instructions. Results are expressed as spot-forming cells (SFC)/10^6 PBMCs in stimulating cultures after subtracting the background. The cut-off value was set by calculating the mean of the background + 2 standard deviation (25 SFC).

Statistical Analysis. The primary analysis of the observational study was to investigate clinical and laboratory characteristics in two groups defined as SARS-CoV2 infected vs. uninfected. Continuous variables were described using median and interquartile ranges, categorical variables using frequencies and percentages. Secondary analysis

was performed to ascertain risk factors associated with SARS-CoV-2 infection in del22q11.2. To evaluate infection prediction performance of lymphopenia (lymphocytes < 1500/mm3), a simple logistic regression model was developed, and odds ratio (OR) and 95% confidence intervals were measured. For the study on immunization, patients have been compared with controls. Immunological and clinical variables were compared between the different study times. Values were compared by the non-parametric Kruskal–Wallis test, and, if not significant, the Wilcoxon matched pair signed-rank test or the two-tailed Mann–Whitney U-test were used. Differences were deemed significant when $P < 0.05$. Statistical analysis was performed with SPSS 18.0 soft- ware for Windows (SPSS, Chicago, IL, USA).

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Results.

Patients. Sixty adults (median age 28 years (IQR:24-35), females 42%) with 22q11.2DS were included in the study. CHD was recorded in 38% of patients, with VSDs and ToF being the most common condition (25% and 14%, respectively). Previous heart surgery was recorded for 30% of patients. Psychiatric conditions were recorded in 65% of participants, neurological conditions in 20%, learning issues in 76%, autoimmune disorders in 42% (3% under immunosuppressive treatment). The analysis of known risk factors for severe COVID-19 identified 56% of patients being overweight, with 25% being obese; moreover 9% of patients had diabetes mellitus type-2 and 15% hypertension (Table 1). Baseline immunological evaluation (Table 2) showed lymphopenia in 38% of patients, with 17% having <400 CD4+ cells/mm3. Moreover, 8% of patients had low IgG serum levels, 16% a selective IgM deficiency (<35 mg/dL) and 4% a selective IgA deficiency (<68 mg/dL).

SARS-CoV-2 infection. Over the study period, 21 patients (35%) (median: 28 years, range: 18-51, females 48%) were diagnosed with SARS-CoV-2 infection (Figure 1). The median duration of qRT-positivity was 10 days (IQR:10-16.5). Twenty patients (95%) did not require hospital admission. The severity of infection was staged as asymptomatic in two patients (9,5%), mild in 18 patients (85,7%) and severe in one patient (4,8%). The most common symptoms were fever (57%), followed by cough (33%), asthenia (29%) and nasal discharge (19%).

Infected patients were analyzed during different periods of the pandemic (Figure 1 and Table 4). Three patients were diagnosed in the pre-vaccination period, from February 2020 to March 2021 (main circulating VOC Wuhan). A 50-years old man affected by DMT2, hypertension, and obesity was diagnosed with SARS-CoV-2 infection seven

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days after being hospitalized for acute kidney failure with nephrosis and secondary hypogammaglobulinemia. He developed COVID-19 acute respiratory distress syndrome and he was admitted to the intensive care unit to receive invasive mechanical ventilation, steroids, heparin and continuous renal replacement therapy. Ten days after the COVID-19 diagnosis the patient died. The other two unvaccinated had a mild course of infection and did not require specific treatment. In March 2021, vaccination against SARS-CoV-2 became available in Italy. In the following months (post-full immunization period), we recorded six cases of SARS-CoV-2 infection. In detail, from March to mid-July 2021 (main circulating VOC Alpha) two immunized patients (two doses) became infected, having an asymptomatic/mild course despite their risk factors. From mid-July to October 2021 (main circulating VOC Delta), four immunized patients (two doses) were infected, all with mild course despite having risk factors for severe COVID-19.

In October 2021, immunization with the booster dose began. In the following months, with the spread of the Omicron variant (post-booster dose period), 12 patients were infected, 10 with mild symptoms and two asymptomatic. Four months after recovery, one patient was re-infected by SARS-CoV-2 with a mild course. At the time of the SARS-CoV-2 infection, 11 patients were immunized with three doses of vaccine, and one patient with four doses (available from January 2022). Seven patients received anti-COVID-19 therapies (Table 4), showing a shorter duration of infection in comparison to untreated patients (10 days (IQR: 10-10) *vs.* 14.5 days (IQR: 10-28.5), p=0,0109).

Compared to uninfected patients, patients infected with SARS-CoV-2 had a more severe degree of intellectual disability (moderate/severe intellectual disability: 62% vs.

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21%, p=0.0020). However, the groups did not differ in age, gender, major CHD, or psychiatric issues.

Moreover, infected patients had a lower pre-infection lymphocytes count (1260 cells/mm3, IQR: 1003-1796 *vs.* 1867 cell/mm3, IQR: 1392-2308, p=0.0055) and lower CD4+ cells count (488 cell/mm3, IQR: 389-656,5 *vs.* 720 cell/mm3, IQR: 539-1000, p=0.0051) (Table 3).

A simple logistic regression confirmed lymphopenia (<1500 cell/mm3) as predictor of SARS-CoV-2 infection (OD 15.7 (95%CI, 3.6-68.9). At the end of the study, 68.3% of patients had been vaccinated with three doses, 13,3% with four doses, 11,7% with two doses, and one patient (1,7%) with only one dose. Three patients (5%) were not vaccinated, including the patient who died before vaccine availability (Figure 2).

Impact of SARS-CoV-2 infection on cardiovascular diseases. Forty percent of patients who recovered from SARS-CoV-2 infection had a major CHD. During SARS-CoV-2 infection, neither patients with CHD nor those without CHD displayed COVID-19 related cardiovascular manifestations, including myocarditis. Only one patient with corrected ToF had transient hypertension. After SARS-CoV-2 recovery, 75% of infected patients underwent transthoracic Doppler echocardiography and EKG at rest (Table 4). None showed echocardiographic or heart rhythm changes compared to pre-SARS-CoV-2 infection. We recorded arterial hypertension in one overweight patient with isolated VSD. Thus, except for the latter patient, no changes in consolidated treatment for cardiovascular conditions were prescribed after recovery.

Immune response to SARS-CoV-2 vaccination. Sixteen patients (age 27 years (IQR:24-36) females 6 (60%) were analyzed for humoral and cellular response to

immunization with the mRNA BNT162b2 (Table S1). To note, two patients with autoimmunity on immunosuppressive treatment were also enrolled: one with rheumatoid arthritis treated by Tocilizumab and one with autoimmune cytopenia receiving steroids. The second patient was also under IgG replacement treatment due to hypogammaglobulinemia.

Following the 2nd vaccine dose, anti-Spike (S1) IgG and IgA increased, even if 22q11.2DS patients showed lower antibody levels than HD (S1-IgG $p<0.0001$; S1-IgA $p=0.0224$). In both groups, anti-S1 antibodies decreased over time and were boosted by the third immunization (S1-IgG: HD $p<0.0001$ and 22q11.2DS $p=0.0020$; S1-IgA: HD $p<0.0001$ and 22q11.2DS $p=0.0020$). Differently from S1-IgG, 22q11.2DS patients reached lower S1-IgA serum levels compared to HD ($p=0.0058$) (Figure 3A-B).

As previously reported (5), we identified low affinity and high affinity MBCs that were detectable respectively positive for PE (S+) or double positive (S++) for PE and BUV395.

At T0 low affinity MBCs (S+) were already detectable in HD and, with lower frequency ($p<0.0001$), in 22q11.2DS patients (Figure 3C). In HD, the frequency of S+ MBCs increased after the second dose ($p=0.0072$), returned to the pre-immunization levels before the 3rd dose ($p<0.0001$) and then augmented after the 3rd dose ($p<0.0001$). In 22q11.2DS patients the frequency of S+MBCs was lower than in HD both after the 2nd ($p=0.0034$) and the 3rd dose ($p<0.0001$) (Figure 3C). We have previously shown that S+MBCs are mostly of IgM-isotype (37). In accordance with our findings, in 22q11.2DS patients the amount of S+MBCs was directly related with the concentration of serum IgM ($R=0.53$, $p=0.0113$).

S++MBCs were absent before immunization both in HD and 22q11.2DS (Figure 3D). After the 2nd dose, the frequency of S++MBCs increased both in HD ($p<0.0001$) and

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362 in patients (p=0.0312). S++MBCs increased after the 3rd dose in HD (p<0.0001) and
363 in 22q11.2DS (p=0.0391), although their frequency was lower in patients (p=0.0161)
364 (Figure 3D). Among total Spike-specific MBCs (S+ plus S++), we also identified RBD-
365 specific MBCs (Figure 3E), a minority of the MBCs generated by vaccination that
366 produce most of the neutralizing antibodies (5). In HD, RBD+ cells increased after the
367 2nd dose (p<0.0001) and even more in the following months (pre 3rd p=0.0090). In
368 22q11.2DS, RBD+ cells expanded after the 2nd (p=0.0489) and the 3rd dose
369 (p=0.0472), with the frequency of RBD+ and MBC S++ cells being directly related
370 (R=0.34, p=0.0371). To note, one patient with hypogammaglobulinemia under IgRT
371 and steroids responded to the booster dose with S+MBC only and did not generate
372 anti-S1 IgG, S++MBCs, and RBD+ cells. Moreover, one patient treated with
373 tocilizumab developed S+MBC but not S++MBC and RBD+ cells, possibly due to
374 impaired germinal center reaction caused by defective IL-6 release (38). We further
375 analyzed the specific T cell-mediated immune responses in 16 patients before and
376 one week after the 3rd vaccine dose by Elispot assay to quantify IFNγ-producing
377 antigen-specific T cells. When stimulated by the full-length Spike WT protein, all
378 patients developed a T-cell response, except for the one treated with steroids (Figure
379 4A). Degrees of response were variable, being directly related to the number of total
380 peripheral lymphocytes (R=0.43, p=0.0310) and CD4+ T cells (R=0.44, p=0.0185).
381 When stimulated by the mutated Spike epitope leading to the Omicron VOC, only 50%
382 of patients responded (Figure 4B). When compared to Omicron Spike responders,
383 nonresponders showed a lower count of lymphocytes (2330 cells/mm3 (IQR:1790-
384 3570) *vs.* 1131 cells/mm3 (IQR:890-2010), p=0.0300). Differently, when stimulated
385 with the same epitope in the WT configuration, all but one patient showed detectable

386 T-response (Figure 4C). This patient was the same who did not respond to the Spike
387 WT protein.
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Discussion

22q11.2DS is the most common chromosomal microdeletion reported in humans (39,40). Besides congenital heart disease, palatal abnormalities, learning difficulties, and neuropsychiatric disorders (41-43), 22q11.2DS is characterized by the absence or under-development of the thymus with impaired immune functions (22,44). The immunological impairment is highly heterogeneous, ranging from a severe combined immunodeficiency phenotype, characterized by profound impairment of T and B cell responses and life-threatening infections, or, more commonly, less severe immune defect with a mild to moderate reduction of T cells and autoimmunity (21-23,44). Antibody deficiencies are also increasingly recognized (24).

Currently, data on the course of SARS-CoV-2 infection in 22q11.2DS patients are scarce and limited to pediatric case reports (9-15) or self-assessment for adults (16), with about 50 cases being reported. Moreover, descriptions of SARS-CoV-2 infections in adults did not include data on infection with the Omicron strain (16). To note, data on immunological response to immunization are lacking.

Published reports of SARS-CoV-2 infection among people with 22q11.2DS revealed both children and adults did surprisingly well, despite their underlying comorbidities (Table 5). In our cohort the course of SARS-CoV-2 infection was free from complications in all but one unvaccinated patient with severe concomitant comorbidities. The symptoms commonly reported were comparable to those experienced by the general population (45). No new cardiovascular impairment during acute SARS-CoV-2 infection or in the post-COVID-19 period nor worsening prior cardiological status was observed except for two patients who developed arterial hypertension. In addition, transthoracic color Doppler echocardiography and EKG at rest performed post-recovery did not detect any new echocardiographic and heart

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rhythm alterations in 22q11.2 infected individuals. This data was entirely unexpected since the European Society of Cardiology has identified adults with CHD as an increased risk population for complications with COVID-19. In particular, cyanotic CHDs, which are frequent cardiological features of people with 22q11.2DS (29), were associated with the high-risk group (46). Moreover, comorbidities commonly found in 22q11.2DS, such as diabetes, hypertension, or being overweight (47) further increase the risk of developing more severe symptoms (47). Cardiological involvement during COVID-19 has also been shown in subjects without heart conditions, with new acute coronary syndromes, arterial and venous thrombosis, acute heart failure, arrhythmias and myocarditis frequently observed (32,48,49). Moreover, the risk for cardiovascular impairment was also increased early after recovery either in those with symptomatic or asymptomatic SARS-CoV-2 infection (50). Compared to uninfected patients, SARS-CoV-2 22q11.21-positive patients had a higher degree of intellectual disability, possibly causing difficulties in keeping social distance and isolation from infected caregivers. Low pre-infection lymphocytes count and reduced CD4+ cells were also found to be associated with a higher risk for infection. It was remarkable that despite having persistent T lymphopenia these patients experienced full clinical resolution of SARS-CoV-2 infection.

The main contributor to the good outcome might be the high immunization coverage recorded in our cohort, with >95% of patients being immunized with at least three doses. This hypothesis is supported by the prospective study on immunological response to SARS-CoV-2 immunization. In 22q11.2DS patients, the IgG responses were comparable to those found in HD, while Spike-specific IgA levels were lower. Moreover, the generation of Spike-specific MBCs and RBD B-cells was slightly impaired as expected, due to the known defect of switched memory in 22q11.2DS

subjects (51,52). Specific T-cell responses were related to total lymphocytes, and CD4+ T cell counts. Recently, low pre-SARS-CoV-2 infection lymphocyte count was confirmed to be an independent risk factor for mortality in a heterogeneous UK cohort of patients with primary and secondary immunodeficiencies (3). In 22q11.2DS patients, mild to moderate lymphopenia represent the primary manifestation of thymic hypoplasia (53) and is more common in infancy than in adulthood (25). However, although patients have reduced T-cell numbers, their repertoire is normal (54). This was confirmed by the observation that lymphopenia does not seem to correlate with the severity and recurrence of infections (20).

In conclusion, our data suggest that vaccination should be encouraged in individuals with 22q11.2 since the mRNA vaccine was able to induce the B/T-cell responses and a robust IgG-specific response. A limitation of the study is the short follow-up time post-immunization. For now, we know that shortly after completing the third dose of vaccine, Spike-specific MBC-response reached lower frequencies than reported in HD, and a subgroup of patients were not able to generate high-affinity specific-MBC, suggesting possible incapability of B-cells to undergo affinity maturation in the germinal center. Previous studies exploring the immunogenicity of vaccinations with live viruses and influenza virus showed that despite the robust seroconversion recorded soon after immunization (26-28), patients with 22q11.2DS have difficulty in sustaining long-term protective antibodies (28). These data together suggest that patients with 22q11.2DS should be periodically re-assessed to identify those needing additional recall vaccine dose administration.

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Figures legends

Figure 1. SARS-Cov-2 infection and immunization coverage in a cohort of 60 patients with 22q11.2DS over the period March 2020 to the end of June 2022. Data on SARS-CoV-2 infection are reported according to the availability of immunization.

Figure 2. Vaccination coverage rate in the enrolled cohort of 60 adults with 22q11.2 DS at the end of study period.

Figure 3. Specific antibody and B-cells response after immunization with two and three doses of mRNA BNT162b2 vaccine. Spike-specific IgG (panel A), Spike-specific IgA antibodies (panel B), S+ (panel C) MBCs, S++ MBCs (panel D) and RBD positive MBCs (panel E) in HD (blue circles) and 22q11.2DS patients (gray circles) before (T0), one week after the second dose (post-2nd dose), six months after the second dose (pre-3rd dose), and one week after the third dose (post-3rd dose) of BNT162b2 vaccine. The MBC subset was defined as CD19 + CD24 + CD27 + CD38-. For Spike-specific IgG and IgA the positive cut-off value was settled at 1.0 OD ratio.

For each group, the median is shown as a bar. Continuous lines represented paired Wilcoxon's test, and dashed lines represented unpaired Mann U Whitney test. Levels of significance: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P < 0.0001$.

Figure 4. SARS-CoV-2-specific T cell responses in 22q11.2DS patients before (6 months after the second dose) and after the third dose of mRNA (BNT162b2) vaccine. The cumulative IFNγ-positive T cell responses (total SFU against complete WT Spike, Omicron Spike, and Omicron WT antigens) were evaluated by the T-SPOT Discovery SARS-CoV-2 ELISpot assay in sixteen virus-naive participants. Statistical analyses

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688 were performed using the Wilcoxon matched-pairs signed-rank test. *P < 0.05, **P <
689 0.01, ***P < 0.001; ****P < 0.0001.

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Table 1. Demographics, clinical characteristics of 60 patients with 22q11.2 DS

	22q11.2DS n=60
Demographics	**n (%)**
Female, n (%)	25 (42)
Age, median (IQR)	27 (24-35)
Clinical data	**n (%)**
BMI	
>25 (overweight)	33 (56)
>30 (obesity)	15 (25)
Cardiovascular conditions	
Previous heart surgery	18 (30)
Major CHD	23 (38)
Hypertension	9 (15)
DMT	4 (9)
Dyslipidemia	13 (22)
Smokers	11 (19)
Psychiatric conditions	39 (65)
Neurological conditions	12 (20)
Learning issues	45 (76)
Autoimmune disorders	25 (42)
Thyroiditis	14 (24)
Psoriasis	5 (8)
Autoimmune cytopenias	3 (5)
Alopecia	2 (3)
Arthritis	1 (2)
Immunosuppressive treatment	2 (3)
Steroids	1 (1,5)
Tocilizumab	1 (1,5)

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Table 2. Immunological data on 60 patients with 22q11.2 DS

	n (%)
Neutropenia (<1000 cell/mm3)	0
Lymphopenia (<1500 cell/mm3), n (%)	20 (38)
IgG <700 mg/dL, n (%)	4 (8)
IgA <68 mg/dL, n (%)	2 (4)
IgM <40 mg/dL, n (%)	8 (16)
CD4+ count (cell/mm3), median (IQR)	585 (478-797)
>=600 cells/mm3, n (%)	20 (49)
400-600 cells/mm3, n (%)	14 (34)
<400 cells/mm3, n (%)	7 (17)
CD8+ count (cells/mm3), median (IQR)	358 (258-494)
<420 cells/mm3, n (%)	24 (61,5)
CD19+ count (cells/mm3), median (IQR)	216 (159-308)
<90 cells/mm3, n (%)	1 (3)

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Table 3. Clinical and demographic characteristics and pre-infection lymphocytes in 22q11.2DS patients SARS-CoV-2 positive and SARS-CoV-2 negative.

	SARS-CoV-2 positive (n=21)	**SARS-CoV-2 negative (n=39)**	p value
Age, median (IQR)	28 (25,5-35,5)	27 (27-34)	0,8025
Female, n (%)	10 (48)	15 (38)	0.5865
Major CHD, n (%)	8 (38)	16 (41)	1,0000
Intellectual disability (moderate/severe), n (%)	13 (620)	8 (21)	0.0020
Psychiatric disease, n (%)	14 (67)	25 (64)	1.0000
Immunological data			
Lymphocytes, median (IQR)	1260 (1003-1796)	1867 (1392-2308)	0,0055
CD4+, median (IQR)	488 (1003-1796)	720 (539-1000)	0.0063
Immunoglobulin defect, n (%)	7 (39)	5 (16)	0.0942

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Table 4. Individual data of 21 patients infected by SARS-CoV-2 during the study time

Period	Sex	Age range	Lymphocytes (cell/mm3)	CD4+ count (cell/mm3)	Igs defect	Risk factors for severe COVID-19	Dose(s) of vaccine at SARS-CoV-2 infection	COVID-19 course	Treatment	Days of qRT PCR positivity	Outcome	Cardiological outcome
Pre-iimmunizzation	M	50-29	1000	380	IgG	DMT2, Obesity, Kidney failure, Mood disorders	None	Severe (ARDS)	O2 therapy, dialysis	NAp	Death	Nap
Pre-iimmunizzation	F	20-29	1290	438	No	Developmental disabilities, Schizophrenia spectrum disorders	None	Mild	None	21	Recovery	No echocardiographic and EKG changes
Pre-iimmunizzation	F	40-49	1500	580	No		None	Mild	Antibiotic	10	Recovery	No echocardiographic and EKG changes
Post-full immunization	M	20-29	820	190	No	Corrected subaortic VSD, multiple VSDs, Schizophrenia spectrum disorders, Developmental disabilities,	Two doses	Asymptomatic	None	10	Recovery	No echocardiographic and EKG changes
Post-full immunization	F	30-39	1230	488	IgG, IgM and IgA defect	Corrected ToF, Overweight, dyslipidemic, Developmental disabilities, Schizophrenia spectrum disorders,	Two doses	Mild	None	10	Recovery	NA
Post-full immunization	F	20-20	1810	NA	No	Small subaortic VSD in natural history	Two doses	Mild	NSAID	17	Recovery	No echocardiographic and EKG changes
Post-full immunization	M	30-39	1180	400	No	Small subaortic VSD in natural history, Overweight, chizophrenia spectrum disorders	Two doses	Mild	None	39	Recovery	No echocardiographic and EKG changes, Arterial Hypertension diagnosis
Post-full immunization	F	40-49	760	270	IgG	Obesity, Developmental disabilities, schizophrenia spectrum disorders	Two doses	Mild	None	16	Recovery	No echocardiographic and EKG changes
Post-full immunization	F	20-29	1690	662	No	Developmental disabilities,	Two doses	Mild	None	31	Recovery	No echocardiographic and EKG changes
Post-booster dose	F	30-39	900	390	No	Developmental disabilities	Three doses	Mild	Paxlovid	10	Recovery	No echocardiographic and EKG changes

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Post-booster dose	F	18-19	1755	720	No	Overweight, Developmental disabilities, Mood disorders	Three doses	Mild	None	10	Recovery	No echocardiographic and EKG changes
Post-booster dose	M	20-29	1180	554	No	Corrected ToF, Overweight, Developmental disabilities, Schizophrenia spectrum disorders	Three doses	Asymptomatic	None	10	Recovery	No echocardiographic and EKG changes
Post-booster dose	F	20-29	1000	400	No	Obesity, Developmental disabilities, Schizophrenia spectrum disorders	Three doses	Asymptomatic	Sotrovimab	7	Recovery	No echocardiographic and EKG changes
Post-booster dose	M	30-29	2030	875	No	Overweight, Developmental disabilities, Schizophrenia spectrum disorders	Three doses	Mild	None	13	Recovery	No echocardiographic and EKG changes
Post-booster dose	m	20-29	2600	1089	IgM defect	Corrected IAA, Overweight, Developmental disabilities,	Three doses	Mild	Sotrovimab	7	Recovery	NA
Post-booster dose	F	20-29	1131	585	IgG/IgA defect	Corrected ToF, Corticosteroids medications	Three doses	Mild	Molnupiravir	10	Recovery	Hypertension during infection. No echocardiographic and EKG changes
Post-booster dose	M	50-59	1650	650	IgM defect	Corrected Hemitruncus, DMT2, Obesity	Four doses	Mild	None (refused)	63	Recovery	No echocardiographic and EKG changes
Post-booster dose	M	20-29	1530	NA	No	Obesity, Developmental disabilities, Schizophrenia spectrum disorders	Three doses	Mild	Sotrovimab	15	Recovery	NA
Post-booster dose	M	30-39	3700	NA	No	Obesity	Three doses	Mild	Paxlovid	7	Recovery	NA
Post-booster dose	M	20-29	2340	550	No	Developmental disabilities, Schizophrenia spectrum disorders	Three doses	Mild	None	NA	Recovery	No echocardiographic and EKG changes
Post-booster dose	M	20-29	1500	NA	No	Developmental disabilities, Schizophrenia spectrum disorders	Three doses	Mild	Molnupiravir	10	Recovery	NA
Post-booster dose	F	20-29	1000	400	No	Obesity, Developmental disabilities, Schizophrenia spectrum disorders	Three doses	Mild	Antibiotic	10	Recovery	No echocardiographic and EKG changes

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Abbreviations: VOC variant of concern, M male, F female, DMT2 Diabetes mellitus type-2, VSD isolated ventricular septal defect, ToF Tetralogy of Fallot, IAA interrupted aortic arch, NA not available, NAp not applicable, NSAID non steroidal anti inflammatory drugs

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Table 5. Published reports of SARS-CoV-2 infection in 22q11.2DS

Ref.	Study design	Infection period considered	Age, sex	Comorbidities	COVID-19 Symptoms	Hospital admission	Treatment	Outcome
(11)	cross-sectional study (prevalence study) in June 2020 (end of first wave) including 65 moderate/ severe IEIs patients	February 2020-Jun 2020	M/17y	Moderate/severe lymphopenia	Asymptomatic	No	No	Recovered
			M/5y	Moderate/severe lymphopenia	Mild (Cough)	No	No	Recovered
(13)	Retrospective survey on 20 IEIs patients	February 2020-September 2020	M/1.5y	Bronchiectasis, Low TRECs level Hypogammaglobulinemia,	Asymptomatic	No	No	Recovered
(1)	A retrospective study was undertaken by a web-based survey, including 94 patients with an underlying IEIs and infected by SARS-CoV-2	March, 2020-June, 2020.	M/ age group 0-2	Lung disease, tracheostomy with chronic ventilation	Severe (Fever, dyspnea)	Yes	Convalescent plasma, O2 support, NIV	Recovered
(18)	Case series of 60 individuals with IEIs National Registry, data collection proformas were sent to all UK pediatric and adult immunologists by email; 100 IEIs patients included	March 2020-July 2020	M/ >18y,	NA	NA	Yes	NA	Recovered
(11)	Cross-sectional, multicenter study, involving 121patients with IEIs	March 2020- December 2020.	M/0,7y	Arterial hypertension, corrected congenital cardiopathy, hypogamma	Severe (fever, cough, dyspnea,severe diarrhea)	Yes (ICU)	NA	Recovered
(14)	Retrospective multicentre survey, 114 IEIs patients included	March 2020-April 2021	F/13.8y	Autoimmune hypothyroidism	Mild (Fever)	No	NA	Recovered
			F/7y	None	Mild (Fever)	No	NA	Recovered
			F/23y	Congenital heart disease, Allergy, Bronchiectasis	Mild (Running nose/Sore throat)	No	NA	Recovered
			F/1.5y	History of interventricular defect and supraventricular paroxysmal tachycardia	Mild (Fever)	No	NA	Recovered
			F/17y	Autoimmune thyroiditis, Interventricular defect	Asymptomatic	No	NA	Recovered
			F/ 9.25y	Congenital heart disease (Interrupted aortic arch, Interatrial defect, Interventricular defect)	Mild (Fever, Asthenia)	No	NA	Recovered

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			M/ 18.4y	Autoimmune thyroiditis, Mild thrombocytopenia,Hyperbilirubinemia, Vitamin D deficiency	Mild (Running nose)	No	NA	Recovered
			F /11.5y	Obesity, Cognitive disability	Moderate (Cough, Headache, Dyspnea, Asthenia, Nausea, Arthralgia)	No	NA	Recovered
			M/10.6y	Left aortic arch, Developmental delay	NA	No	NA	Recovered
			M/1.6y	Hypoparathyroidism, Hypocalcemia, Congenital hypothyroidism, Bicuspid aorta	Asymptomatic	No	NA	Recovered
			F/15.2y	Hyperthyroidism, Intellectual disability, Congenital heart disease	NA	No	NA	Recovered
			M/1y	Minimal left to right interatrial shunt, nephrocalcinosis, hypoparathyroidism, Areas of parenchymal lung consolidation, hypogammaglobulinemia	Severe (fever)	Yes	NA	Recovered
(3)	National Registry, data collection proformas were sent and 310 patients included	March 2020- July 2021	Two males (18 and 21years)	NA	Severe course (one patient)	Yes (one)	NA	Recovered
(10)	Cross-sectional, multicenter study involving 99 IEIs patients	June 2020 - June 2021	M/1,5y	NA	Mild (Anxiety, Bradycardia)	NA	NA	Recovered
(12)	Case report	NA (published on feb 2021)	F/12y	Hypogammaglobulinemia, T cell lymphocytopenia, congenital heart disease, and VP shunt	Mild (headache, emesis).	NA	No	Recovered
		NA (published on feb 2021)	M/13y	obesity, and congenital heart disease, low IgM.	Asymptomatic	NA	NA	Recovered
(15)	Multinational survey on 152 patients with 22q11.2DS	July 2021-December 2021	25 patiens, (range 2–36 years)	Overweight 21% Ig infusions 4% Previous heart surgery 43% Hypertension 4% Psychiatric problems 11% Arthritis 11%, Asthma 29%	Fatigue (63%), headache (54%), cough (51%), rhinorrhea (45%)	1/25 hospitalized for one day.	None	25/25 Recovered

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Abbreviation: 22q11.2DS 22q11.2 deletion syndrome; IEIs inborn errors of immunity, NA not available, M male, F female, Y years, NIV not invasive ventilation, O2 oxygen, ICU intensive care unit, SARS-CoV-2 severe acute respiratory syndrome coronavirus 2, TRECs T-cell receptor excision circles

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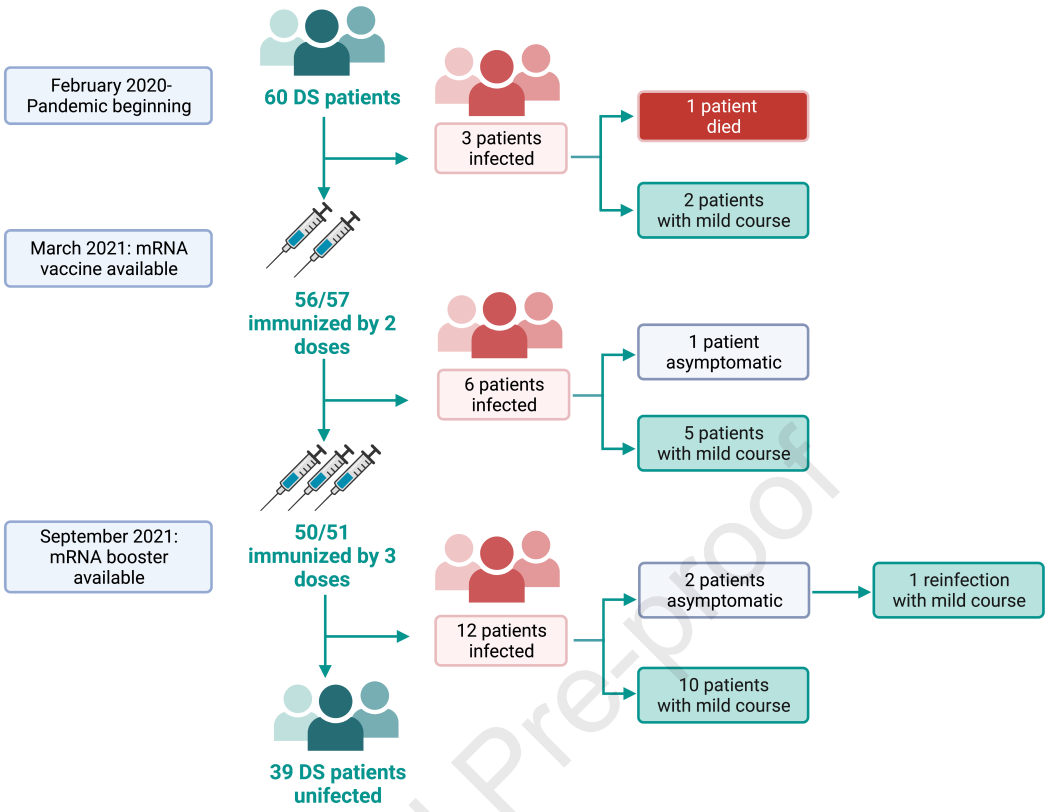

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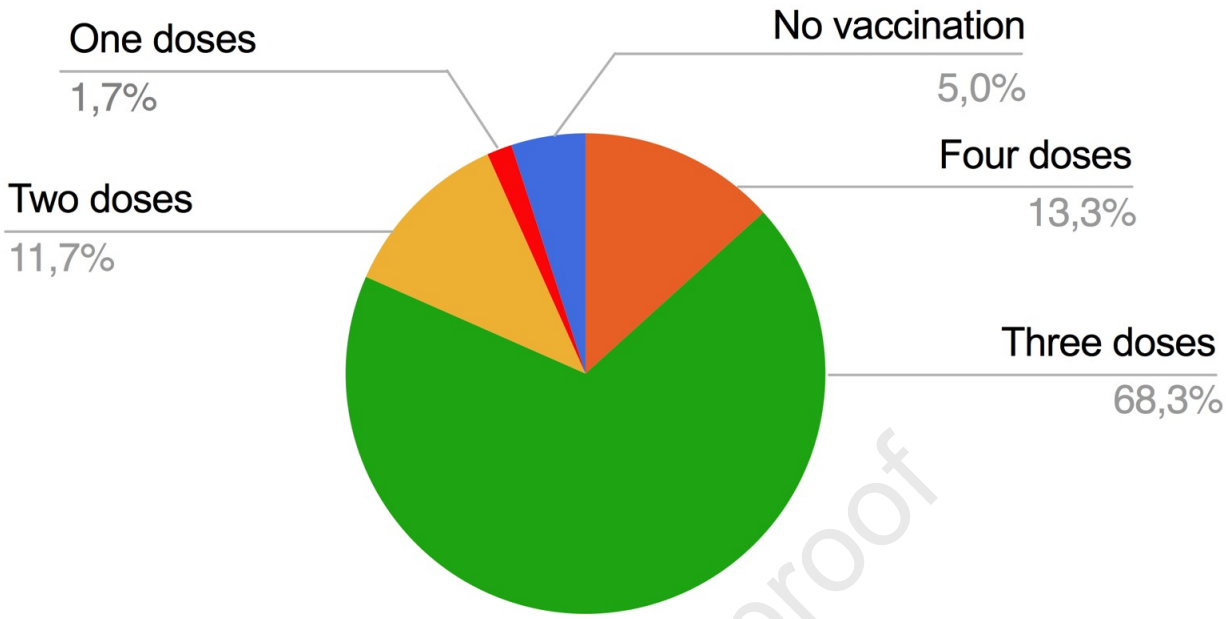

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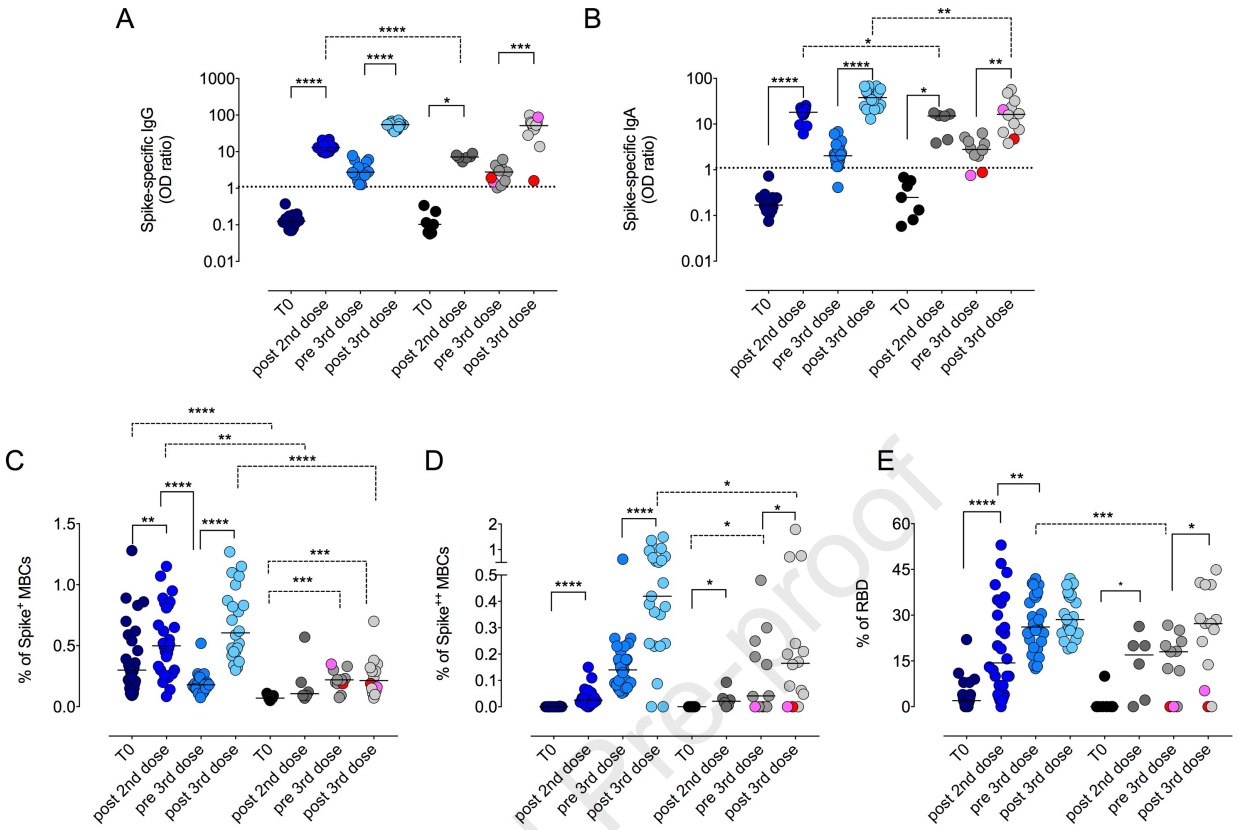

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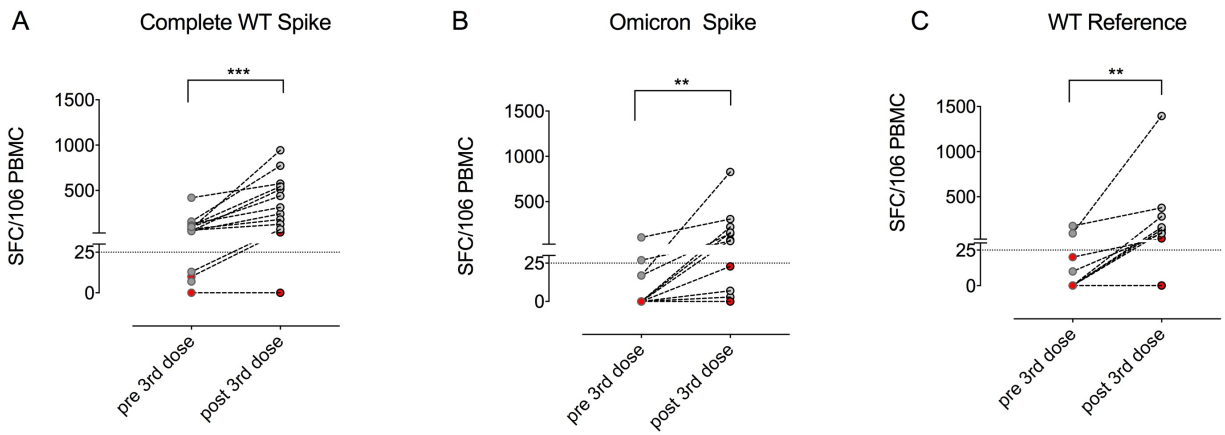

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