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**Abstract:** Our goal was to determine the cellular immune response (CIR) among a random sample of the Borriana COVID-19 cohort (Spain) to identify its associated factors and their relationship with infection, reinfection and sequelae. We conducted a nested case-control study using a randomly selected sample of 225 individuals age 18 and older, including 36 individual naïve to SARS-CoV-2 infection, and 189 infected patients. We employed flow cytometry-based immunoassays for intracellular cytokine staining, utilizing Wuhan and BA.2 antigens and chemoluminescence microparticle immunoassay for detection SARS-CoV-2 antibodies. Logistic regression models were used. A total of 215 (95.6%) participants exhibited T-cell response (TCR) to at least one antigen. Positive responses of CD4+ and CD8+ T cells were 89.8% and 85.3% respectively. No difference in CIR was found for naïve and infected patients. Patients who experienced sequelae exhibited higher CIR than those without. A positive correlation was observed between TCR and Anti-Spike IgG levels. Factors positive associated with TCR included A-blood group, number of SARS-CoV-2 vaccine doses received, and Anti-N IgM; factors inversely related were the time elapsed since the last vaccine dose or infection, and B-blood group. These findings contribute valuable insights into the nuance immune landscape shaped by SARS-CoV-2 infection and vaccination.

**Keywords:** COVID-19; SARS-CoV-2 infection; cellular immunity; T cells; CD4+, CD8+, humoral immunity; Anti-S antibodies Anti-N antibodies; nested case-control; associated factors;

**1. Introduction**

Three years have passed since the SARS-CoV-2 infection pandemic began, leading a roughly 6.900.000 million fatalities globally; over half of the world's population has been infected, and a significant number of those infected are suffering long-term sequelae, and the virus continue to be a persistent threat [1]. Research on SARS-CoV-2 strains` evolution, the pandemic`s progression, and its impact on human health remains critical. This includes studying the immune response to the infection and vaccinations. Such research is essential for guiding health strategies and medical interventions [2,3].

The immune response to SARS-CoV-2 infection is characterized by humoral and cellular immunities [4]. Research indicates that specific CD4+ and CD8+ T cells are linked to less severe forms of the disease [5–7]. SARS-CoV-2 vaccines effectively reduce the severity and hospitalization rates caused by the virus. However, this effectiveness against infection and transmission is somewhat lower. The vaccine`s protection against severe outcomes is largely attributed to cellular immunity response [8,9]. The study of cellular immunity is crucial for understanding protection against SARS-CoV-2, emerging variants and the developing new vaccines [10,11]. Testing cellular immunity protection is challenging due to the complexity, laborious natures and specialized techniques required to understand the biological mechanisms involved [12].

The research on cellular immune response to SARS-CoV-2 has focused mainly on patients with severe outcomes, those hospitalized or suffering from long-SARS-CoV-2 infection, and immune-compromised individuals, with less emphasis on unselected populations [13]. Our investigation began with the SARS-CoV-2 outbreak at the Falles Festival in March 2020, with 570 cases among 1332 attendees in Borriana, Valencia Community (Spain). Since then, we have conducted various follow-up studies [14–16]. In the latest follow-up, we analyzed a randomly selected sample form the Borriana COVID-19 cohort. This sample encompassed individuals who were either naïve to SARS-CoV-2 infection or had experienced reinfection. We aimed to determine the dynamics between SARS-CoV-2 infection, its clinical outcomes, and the associated cellular and humoral immune response. We also sought to understand the effects of SARS-CoV-2 vaccination and other relevant factors in an unselected population.

**2. Materials and Methods**

*2.1. Explanation*

This cohort was previously studied three times. The first study was conducted in May 2020 with 1332 participants [14]. The second study was conducted in October 2020 with only SARS-CoV-2 infected patients including 484 participants [17]. The third study was conducted in June 2022 and included 722 participants who had undergone at least one laboratory test to verify their SARS-CoV-2 infection status [18].

In December 2022, we conducted a nested case-control study within the Borriana COVID-19 cohort on a randomly selected sample of 225 subjects 18 years old or older from the third study based on the following criteria: 1) a 1:4 ratio of naïve participants to SARS-CoV-2 infection case, 2) a difference of 13% in cellular immune response between the two groups with a power of 80% and alpha error of 5% , following research comparing naïve and SARS-CoV-2 infection patients   [19–21]. The final theory sample included 45 naïve (never infected) participants and 180 SARS-CoV-2 infected patients. In addition, we estimated from our previous data that of 33% SARS-CoV-2 infection patients would experience post-COVID-19 sequelae , and there would be a 20% chance of SARS-CoV-2 reinfection in the third study [22]. A sample size of 103 was calculated for participants with sequelae and 89 for participants with reinfection based on a precision of 5% and power 90%. These estimations were made with Stata ®14 version 2 and OpenEpi (https://www.openepi.com) programs.

The study groups were divided into four categories: 1) naïve participants,2) SARS-CoV-2 infected patients, 3) SARS-CoV-2 infection with sequelae or without sequelae, and 4) SARS-CoV-2 infection reinfections or no-reinfections.

In all 225 subjects, we obtained blood samples to perform the following determinations:

- -Anti-SARS-CoV-2 S spike (IgG), N nucleocapsid (IgG), and N nucleocapsid (IgM) antibodies were estimated by chemoluminescence microparticle immunoassay (CMIA) (Alinity Ι Anti-SARS-CoV-2 S and N, Abbot Laboratories, Chicago, USA) [23]. All these tests were performed at the Microbiology Service Laboratory of the University Hospital de la Plana, Vila-real.
- -Cell immunity against the Wuhan and Omicron BA.2 variants of concern (VOC) were measured with flow cytometry. Functional cellular assays were based on the detection of markers of T-cell activation. Enumeration of SARS-CoV-2-S-reactive interferon-γ-producing CD4+ and CD8+ T cells in fresh heparinized peripheral whole blood was carried out by flow cytometry immunoassay for intracellular cytokine staining (BD Fastimmune, Becton Dickinson and Company-Biosciences, San Jose, CA) as previously described [21,24–26]. Specimens were analyzed at the Microbiology Service, Clinic University Hospital, INCLIVA Health Research Institute, Valencia, (Spain).
- -25-hydroxy vitamin D [25(OH)D] levels by electrochemiluminescence-based assay (Elecsys vitamin D total II Roche Diagnostic, Germany)[27].This analysis was performed at the University Hospital de la Plana Clinical Laboratory Service, Vila-real.

In June 2022, we ran a questionnaire in collaboration with the health staff of the Hospital de la Plana, Castellon Public Health Center, and the health centres of Borriana, Vila-real, Onda, and La Vall d'Uixo. The interviews were conducted by phone or face-to-face, and we collected information about the participants` demographic characteristics, chronic diseases, health habits, body mass index (BMI) (kg/m²), occupation, level of physical exercise, smoking habits, consumption of alcohol, chronic illnesses and SARS-CoV-2 infection exposures, laboratory-confirmed of SARS-CoV-2 incidence and reinfection,   SARS-CoV-2 infection sequelae. To obtain information about SARS-CoV-2 vaccination, we consulted the Register of Vaccination of the Valencia Community for data on administration date, vaccine type, and brand. Finally, we questioned those vaccinated on vaccination adverse effects. Subsequently, in December 2022, at the time of the blood sampling, we ran an additional short questionnaire to ascertain SARS-CoV-2 infection, reinfection and sequelae.

We defined reinfection as a new SARS-CoV-2 infection more than 60 days after a previous SARS-CoV-2 infection and confirmed by polymerase chain reaction test (PCR) or a rapid antigen test (RAT)[28].The first infection could be confirmed by PCR, RAT, or positive Anti-Nucleocapsid IgG determination. We considered that the subject suffered post-SARS-CoV-2 infection sequelae if we obtained a positive response:

"Do you have some attributable sequelae by COVID-19 disease?" following the questionnaire used in June 2022.

### 2.2. Statistical Methods

We employed the following descriptive statistics to explain our results: the mean, standard deviation, median, and ranks. We use the Chi-square and Fisher's exact tests to compare qualitative data. We use Kruskal-Wallis and median tests for quantitative data. We used the Spearman correlation non-parametric coefficient (rs) to analyze the strength and direction of the association between anti-S IgG levels and cellular immunity across the study groups with the study groups. The rs coefficient, ranging from -1 to +1 indicates a perfect positive or negative rank correlation at its extremes, and zero denotes no correlation.

The nested case-control comprised three separate studies: 1) SARS-CoV-2 infected patients (cases) versus naïve participants (controls); 2) SARS-CoV-2 infected patients with sequelae (cases) versus patients without sequelae (controls); 3) Third, SARS-CoV-2 patients with reinfection (cases) versus no-reinfection (controls).

The variable dependent was the cellular immune response measured by production of CD+4 and CD+8 T cells stimulation with Wuhan and BA.2 (Omicron) antigens. We defined a positive response as any percentage response above zero, contrasting with a zero-percentage indicating a negative response. Accordingly, we ran three nested case-control studies to contemplate the impact on the immune response considering the difference experiences in the subjects of our sample: 1)

SARS-CoV-2 infection patients (cases) versus naïve participants (controls); 2) SARS-CoV-2 infection patients with sequelae (cases) versus patients without sequelae (controls); 3) Third, SARS-CoV-2 patients with reinfection (cases) versus no-reinfection (controls). We calculate odds ratios (OR) with a 95% confidence intervals (95% CI) to compare cases and controls. The total of the sample was used to study potential factors associated with the cellular immune response.

We identified potential confounders such as age, sex, time since SARS-CoV-2 infection or reinfection, or since the last SARS-CoV-2 vaccine dose, body mass index, smoking status, alcohol consumption, habitual physical exercise, chronic diseases, and number of anti-SARS-CoV-2 vaccine doses, using Directed Acyclic Graphics (DAGs)[29].Time since SARS-CoV-2 infection or reinfection, or since the last SARS-CoV-2 vaccine dose was included considering that time since these events could play a role in the immune response. We employed multivariate analysis logistic regression models for confounder adjustment.   We conducted our statistical analyses using the program Stata® 14 version 2.

To ensure the robustness of our finding, we undertook a sensitivity analysis. This analysis was based on a definition of a positive immune response   $\geq 0.10\%$ for CD+4 and CD+8 T cells, following the methodology outlined by Gimenez an co-authors [24] . This approach allowed us to affirm the consistency of our results under varying criteria for immune response.

This study was approved by the Ethics Committee of the University Hospital de la Plana in Vila-real. All participants provided written informed consent.

## 3. Results

From the initial pool of 619 participants aged 18 years and older, we obtained a random sample of 225 individuals. This sample was categorized based on SARS-CoV-2 infection status; nine individuals were infected for the first time between June and December 2022. The final sample included 36 naïve to SARS-CoV-2 infection, and 189 previously infected. With the group of previously infected participants, we identified two non-exclusive subgroups: those who developed sequelae following the infection (n=77) and those who did not (n=103), along with participants who experienced reinfections (n=78) and those with a single infection event (n=88). In the previously infected group, details on sequelae were missing for 9 individuals and 23 cases of reinfection were not laboratory-confirmed.

The demographic and clinical characteristic of our study population are detailed in Table 1. The average age varied across groups, with naïve group having average age of 49.5 years and the no-sequelae group 41.0 years. The naïve group was older that the SARS-CoV-2 infection patients (p=0.010), and the patients with sequelae group older than patient without sequela group (p=0.01). Female participation was more prevalent than male in all groups from 55.6% in the naïve group to 64.9% in the SARS-CoV-2 patients with sequelae.

The interval since the last vaccine dose or infection showed minor variations with 10.3 months for patients without reinfection and 11.4 months in the naïve group. Body Mass Index remained fairly consistent across all groups, ranging from 26.4 to 26.9 kg/m². Smoking habits was significant higher in the naïve group comparing with the infected patients 44.4% versus 28.2% (p=0.001).

Alcohol consumption showed minimal variation among the groups. Physical exercise was most frequent in patients without sequelae (64.1%) and least common in the naïve group (50%). The prevalence of chronic diseases was higher in patients with sequelae (44.2%) compared to those without sequelae (33.0%)

Status vaccination rates varied across the groups: 96.8% of infected patients had received at least one dose of a SARS-CoV-2 vaccine. In contrast, 100% of the naïve group had received three vaccine doses compared to 77.8 % in the infected group (p=0.001). The patients with sequelae had received more SARS-CoV-2 vaccine doses than the patients without sequelae (p=0.034). However, the reinfection group had received lower vaccine doses than the no-reinfection group (p=0.028).

Anti-S IgG levels were highest in the no-reinfection group and lowest in the reinfection group (p=0.005). The reinfection group also exhibited higher positivity for Anti-N IgM and IgG compared

to no-re-infected group (p=0.003). Positivity for Ant-N IgG was observed in 56.6% of infected patients.

Vitamin D levels were highest in the sequelae group and lowest in the naïve group. Regarding ABO blood groups, O-group was most frequent in the naïve group and least in the sequelae group. The A-group was more prevalent in the no-reinfection group and least in the reinfection group. The B-group showed a higher prevalence in the sequelae group and lower in the without sequelae. The AB-blood group was most common among naïve participants.

**Table 1.** Distribution of variables in the groups: naïve participants and SARS-CoV-2 infected patients, SARS-CoV-2 patients with sequelae/ without sequelae, and SARS-CoV-2 patients with reinfection/no-reinfection. Borriana COVID-19 cohort 2022.

| Groups | Naïve | Infected SARS-CoV-2 | Sequelae[1] | | Reinfection[2] | |
|---|---|---|---|---|---|---|
| | Controls n=36 | Cases n=189 | Cases Sequelae n=77 | Controls No sequelae n=103 | Cases Reinfection n=78 | Controls No reinfection n=88 |
| Variables | | | | | | |
| Age±SD[3] | **49.5±12.8**\*\* | **43.3±14.3** | **46.5±12.9**\*\* | **41.0±15.0** | 41.8±13.8 | 45.3±13.7 |
| Male (%) | (44.4) | (37.6) | (35.1) | (40.8) | (38.5) | (35.2) |
| Time (months)[4] | 11.4±0.7 | 10.8±3.5 | 10.3±3.5 | 11.1±3.6 | 10.3±4.02 | 11.1±3.4 |
| Body mass index | 26.9±5.9 | 26.7±5.1 | 26.6±4.9 | 26.8±5.1 | 26.5±4.9 | 26.8±5.1 |
| Smoking | **16 (44.4)**\*\* | **38 (28.2)** | 19 (25.0) | 15 (14.6) | 13 (16.7) | 23 (26.4) |
| Alcohol consumption | 28 (77.8) | 139 (73.9) | 58 (75.3) | 75 (73.5) | 58 (75.3) | 67 (76.1) |
| Physical exercise | 18 (50.0) | 112 (59.3) | 40 (52.0) | 66 (64.1) | 49 (62.8) | 50 (56.8) |
| Chronic Disease | 12 (33.3) | 73 (38.6) | 34 (44.2) | 35 (33.0) | 29 (37.2) | 37 (40.1) |
| Vaccinated[5] | 36(100) | 183 (96.8) | 77(100) | 97(94.2) | 74(94.9) | 87(98.9) |
| mRNA[6] only | 25 (69.4) | 138 (75.4) | 57 (74.0) | 74 (76.3) | 54 (73.0) | 68 (78.2) |
| mRNA+others | 11 (30.6) | 45 (24.6) | 20 (26.0) | 23 (23.7) | 20 (27.0) | 19 (21.8) |
| 3 doses (%) | **36 (100)**\*\* | **126(77.8)** | **53 (68.8)**\* | 68 (66.0) | **44 (56.4)**\* | **63 (71.6)** |
| 2 doses | 0 | 53 (28.0) | 24 (31.2) | 25 (24.3) | 26 (33.3) | 24 (27.7) |
| 1 doses | 0 | 4 (2.1) | 0 | 4 (3.9) | 4 (5.1) | 0 |
| 0 doses | 0 | 6 (3.2) | 0 | 6 (5.3) | 4 (5.1) | 1 (1.1) |
| Humoral immunity | | | | | | |
| Anti-S IgG[7] AU/ml | 1986±1627 | 2072±1689 | 2080±1725 | 1892±1658 | **1620±1429**\*\* | **2433.6±1889** |
| Anti-N IgG or IgM[8] | 0 (0) | 116 (61.4) | 44 (57.1) | 68 (66.0) | **56 (71.8)**\*\* | **42 (47.7)** |
| Anti-N IgM | 0 | 25 (13.2) | 9 (11.7) | 16 (15.5) | 14 (18.0) | 8 (9.1) |
| Anti-N IgG | 0 | 107 (56.6) | 41 (53.3) | 62 (60.2) | **52 (66.7)**\*\* | **39 (44.3)** |
| Vit D[9] | 27.0±10.4 | 30.4±9.8 | 31.2±10.6 | 30.1±9.4 | 30.2±10.4 | 30.5±9.3 |

| | | | | | |
|---|---|---|---|---|---|
| Vit D ≥30 ng/ml | 13 (36.1) | 86 (45.5) | 41 (53.3) | 43 (41.8) | 34 (43.6) | 44 (50.0) |
| ABO blood groups | | | | | | |
| O | 14 (58.9) | 80 (42.3) | 28 (36.4) | 47 (45.6) | 34 (43.6) | 35 (40.0) |
| A | 18 (50.0) | 88 (46.6) | 37 (48.1) | 49 (47.6) | 36 (46.2) | 45 (51.1) |
| B | 2 (5.6) | 17 (8.0) | 10 (13.0) | 5 (4.9) | 6 (7.7) | 8 (9.1) |
| AB | 2 (5.6) | 2 (2.1) | 2 (2.6) | 2 (1.9) | 2 (2.6) | 0 |

[1] Missing information n=9. [2] No confirmation tests n=23. [3] SD = standard deviation. [4] Times since the last vaccine dose/infection. [5] One or more doses of any of the SARS-CoV-2 vaccines. [6] Messenger RNA vaccine. [7] Antibodies IgG Spike. [8] Total Antibodies IgG N. [9] Vitamin D. * p-value<0.05. ** p-value <0.01.

The distribution of the cellular immune response is shown in Table 2. The median frequency of positivity presented variations against Wuhan antigen: the immune response of CD8+ T cells varied from 0.11% (0%-13.5%) in no-reinfection group to 0.07% (0%-6.9%) in reinfection group; CD4+ T cells varied from 0.10% (0%-1.05%) in the naïve group to 0.07% (0%-1.47%) in the reinfection group. Against BA.2 antigen, the immune response of CD8+ T cells varied from 0.11% (0%-6.5%) in the naïve group to 0.07% (0%-13.3%) in patients without sequelae; CD4+ T cells varied from 0.13% (0%-0.85%) in the naïve group to 0.07% (0%-1.67%) in the patients without sequelae.

Comparing percentages of positive immune response by groups, the naïve group showed higher response for CD4+ and CD8+ T cells against Wuhan and BA.2 antigens than infected patients. In addition, the SARS-CoV-2 group with sequelae showed also more elevated immune response than the without sequelae group. In contrast, the SARS-CoV-2 reinfection and no-reinfection groups presented an immune response similar.

**Table 2.** Distribution of cellular immune response in naïve group, SARS-CoV-2 infected patients, SARS-CoV-2 patients with sequelae /without sequelae, and reinfection/no-reinfection groups measured by CD4+ and CD8+ T cell response against Wuhan and BA.2 antigens. Borriana COVID-19 cohort 2022.

| | Controls Naïve | Cases Infected Patients | Patients Sequelae Cases | Patients No sequelae Controls | Patients Reinfection Cases | Patients No reinfection Controls |
|---|---|---|---|---|---|---|
| T cell response | Frequency median and range (percentages) [1] | | | | | |
| CD8+ for BA.2 [1] | 0.11% (0%-6.5%) | 0.08% (0%-13.3%) | 0.09% (0%-8.82%) | 0.07% (0%-13.3%) | 0.08% (0%-2.57%) | 0.10% (0%-13.3%) |
| CD4+ for BA.2 [1] | 0.13% (0%-0.85%) | 0.09% (0%-2.37%) | 0.11% (0%-2.37%) | 0.07% (0%-1.67) | 0.09% (0%-2.37%) | 0.10% (0%-1.67%) |
| CD8+ for Wuhan [1] | 0.10% (0%-3.56%) | 0.09% (0%-13.5%) | 0.10% (0%-3.25%) | 0.08% (0%-13.5%) | 0.07% (0%-6.90%) | 0.11% (0%-13.5%) |
| CD4+ for Wuhan [1] | 0.10 (0-1.05) | 0.08% (0%-2.03%) | 0.08% (0%-1.15%) | 0.08% (0%-2.03%) | 0.07% (0%-1.47%) | 0.09% (0%-2.03%) |
| | Numbers of positive (percentages) | | | | | |
| CD8+ for BA.2 [2] | 28(77.8%) | 130(68.8%) | 58(75.3%) | 67(65.0%) | 53(68.0%) | 61(69.3%) |
| CD4+ for BA.2 [2] | 31(86.1%) | 144(76.2%) | 67(87.0%) | 69(67.0%) | 60(76.9%) | 67(76.1%) |
| CD8+ for Wuhan [2] | 29(80.6%) | 136(72.0%) | 61(79.2%) | 68(66.0%) | 52(66.7%) | 66(75.0%) |

| | | | | | |
|---|---|---|---|---|---|
| CD4+ for Wuhan[2] | 32(88.9%) | 146(77.3%) | 69(89.6%) | 72(69.9%) | 59(75.6%) | 70(79.6%) |

[1]Medians (range) CD8+ and CD4+ T cell response against BA.2 and Wuhan variants. [2] Number and positive percentage CD8+ and CD4+ T cell response against BA.2 and Wuhan variants.

In total, 215 (95.6%) participants presented positive cellular immune response to at least against one antigen, and 10 participants (4.4%) had no response. Immune response of CD4+ T cells against Wuhan antigen was 79.2% and against BA.2 was 77.8%, and immune response of CD8+ T cells against Wuhan 73.3% and against BA.2 70.2%. Comparing the naïve and infected patients, the naive group had higher immunity responses, but these differences were not significant. As total, CD4+ T cells responders were 202 participants (89.8%) and no-responders 23 (10.2%) and CD8+ T cells responders were192 participants (85.3%) and no-responders 33 (14.7%). Of the note, the cellular immune response of the naïve group is due to the three doses of the SARS-CoV-2 vaccines.

The cellular immune responses comparing the studied groups are shown by a crude analysis in Table 3. The SARS-CoV-2 infection patients group showed a lower immune response than the naïve group for Wuhan and BA.2 antigens but it was not significant. The SARS-CoV-2 patients with sequelae comparing with patients without sequelae showed significant higher immune responses against Wuhan antigen,   CD4+ T cells OR=3.71 (95% CI 1.60-8.64), and BA.2 antigen, CD4+ T cells OR=3.20 (95% CI 1.51-7.31). However, immune responses against Wuhan and BA.2 antigens by CD8+ T cells were marginally or not significant respectively.  Immune responses of SARS-CoV-2 reinfection patients comparing with no-reinfection patients were not significant.

**Table 3.** Cellular immune response comparisons among the study groups: naïve versus SARS-CoV-2 infected patients, SARS-CoV-2 patients with sequelae/ without sequelae, and SARS-CoV-2 reinfection patients/no-reinfection by logistic regression. Crude analysis. Odds ratio (OR) and 95% Confidence Interval (CI). Borriana COVID-19 cohort 2022.

| | CD8+ for BA.2 | CD4+ for BA.2 | CD8+ for Wuhan | CD4+ for Wuhan |
|---|---|---|---|---|
| | OR 95% CI | OR 95% CI | OR 95% CI | OR 95% CI |
| Infected patients Cases | 0.63 (0.77-1.46) | 0.52(0.19-1.41) | 0.62 (0.25-1.50) | 0.42(0.14-1.27) |
| Naïve   Controls | 1.00 | 1.00 | 1.00 | 1.00 |
| Patients with sequelae Cases | 1.64(0.85-3.17) | **3.20 (1.51-7.31)** | 1.96 (0.99-3.89) | **3.71(1.60-8.64)** |
| Patients without-sequelae Controls | 1.00 | **1.00** | 1.00 | **1.00** |
| Patients reinfection Cases | 0.93(0.49-1.81) | 1.01(0.51-2.15) | 0.67(0.34-1.31) | 0.80(0.38-1.66) |
| Patients no-reinfection Controls | 1.00 | 1.00 | 1.00 | 1.00 |

The cellular immune response comparisons are shown by an adjusted analysis in Table 4. The SARS-CoV-2 infected patients showed lower immune response than the naïve group for Wuhan and BA.2 antigens, except Wuhan CD8+ T cells but again the difference were not significant. The patients with sequelae showed higher significant immune response than the without sequelae group against Wuhan antigen, CD4+ T cells (OR=3.90 95% 1.50-9.52), CD8+ T cells (OR=2.33 95% CI 1.03-5.30), and BA.2 antigen, CD4+ T cells 2 (OR=4.20 95% CI 1.76-10.0). The SARS-CoV-2 reinfection patients showed no significant immune response against Wuhan and BA.2 antigens comparing with no-reinfection group.

**Table 4.** Cellular immune response comparisons among the study groups: naïve versus SARS-CoV-2infected patients, SARS-CoV-2 patients with sequelae/ without sequelae, and SARS-CoV-2 reinfection patients/ no-reinfection by logistic regression. Adjusted odds ratios (aOR). 95% Confidence interval (CI). Borriana COVID-19 cohort 2022.

| Groups | CD8+ for BA.2 | CD4+ for BA.2 | CD8+ for Wuhan | CD4+ for Wuhan |
|---|---|---|---|---|
| | % positive | % positive | % positive | % positive |
| Infected Patients Cases | 68.8% | 76.2% | 72.0% | 77.3% |
| Naïve Controls | 77.8% | 86.1% | 80.6% | 88.9% |
| aOR[1] (96% CI) | 0.81(0.32-2.08) | 0.39(0.13-1.19) | 1.01(0.37-2.77) | 0.45(0.14-1.49) |
| p-value | 0.668 | 0.097 | 0.982 | 0.191 |
| Patients with sequelae Cases | 75.3% | 87.0% | 79.2% | 89.6% |
| Patients without sequelae Controls | 65.1% | 67.0% | 66.0% | 69.9% |
| aOR (95% CI) | 1.24(0.59-2.62) | **4.20 (1.76-10.0)** | **2.33(1.03-5.30)** | **3.90 (1.50-9.52)** |
| p-value | 0.569 | 0.001 | 0.043 | 0.004 |
| Patients reinfection Cases | 67.0% | 76.0% | 66.7% | 75.6% |
| Patients no-reinfection Controls | 69.3% | 76.1 | 75.0% | 79.6% |
| aOR (95% CI) | 0.94 (0.44-1.47) | 0.80 (0.36-1.78) | 0.84(0.38-1.89) | 0.80 (0.35-1.85) |
| p-value | 0.860 | 0.584 | 0.659 | 0.602 |

[1]Adjusted for age, sex, blood groups, body mass index, chronic disease, smoker, alcohol consumption, physical exercise, vaccine doses, time since the last vaccine dose / infection.

The crude analysis of the cellular immune response and associated factors are shown in Table 5. CD8+ T-cell response against Wuhan antigen was linked with alcohol consumption, A-group, and inversely linked with B-group. CD4+ T-cell response against Wuhan was inverse linked with physical exercise. CD8+ T-cell response against BA.2 antigen were associated with age, three vaccine doses versus 0-2, number vaccine doses, Anti-N IgM, and inversely associated with time since the last vaccine dose or infection. CD4+ T cells response was associated with body mass index.

**Table 5.** Cellular immune response against Wuhan and BA.2 antigens and associated factors by logistic regression. Total sample. Crude analysis. Odds ratio (OR) and 95% Confidence Interval (CI). Borriana COVID-19 cohort 2022.

| | CD8+ for BA.2 | CD4+ for BA.2 | CD8+ for Wuhan | CD4+ for Wuhan |
|---|---|---|---|---|
| Variables | OR 95% CI | OR 95% CI | OR 95% CI | OR 95% CI |
| Age | **1.03 (1.0-1.05)** | 1.01(0.93-1.03) | 1.01(0.98-1.03) | 1.01(0.98-1.03) |
| Male (%) | 1.09(0.60-1.96) | 0.84(0.44-1.58) | 1.24(0.67-2.29) | 0.81(0.42-1.57) |
| Time (months)[1] | **0.89(0.81-0.98)** | 0.95(0.86-1.05) | 1.05(0.95-1.15) | 1.07(0.97-1.18) |
| Body mass index (kg) | 1.02(0.96-1.08) | **1.08(1.01-1.15)** | 1.04(0.98-1.10) | 1.02(0.95-1.09) |
| Smoking | 0.81(0.49-1.81) | 0.67(0.33-1.36) | 0.83(0.42-1.63) | 0.69(0.34-1.41) |

| | | | | |
|---|---|---|---|---|
| Alcohol consumption | 0.99(0.51-1.92) | 1.04(0.57-2.13) | **1.98(1.04-3.79)** | 1.04(0.50-2.19) |
| Physical exercise | 0.72(0.41-1.31) | 0.87(0.45-1.65) | 0.78(0.13-1.43) | **0.50(0.25-0.99)** |
| Chronic Disease | 1.64(0.89-3.02) | 1.23(0.63-2.39) | 1.07(0.57-1.97) | 1.78(0.88-3.60) |
| Vaccine anti-SARS-CoV-2 | | | | |
| mRNA homologous vaccine | 1.39(0.72-2.67) | 0.96(0.46-2.01) | 1.05(0.53-2.10) | 0.80(0.36-1.73) |
| 3 doses versus 0,1,2 vaccine | **1.88(1.02-3.48)** | 1.13(0.57-2.26) | 1.42(0.75-2.69) | 0.74(0.35-1.57) |
| Number vaccine doses | **1.70(1.12-2.60)** | 1.15(0.73-1.82) | 1.41(0.92-2.15) | 1.10(0.68-1.38) |
| Humoral immunity | | | | |
| Anti-S IgG AU/ml | 1.00(0.99-1.00) | 1.00(0.94-1.10) | 1.00(0.99-1.00) | 1.01(0.94-1.10) |
| Anti-N IgM or IgG | 1.04(0.54-1.85) | 1.08(0.58-2.03) | 1.19(0.66-2.15) | 0.92(0.48-1.75) |
| Anti-N IgM | **5.54(1.27-24.2)** | 1.16(0.41-3.27) | 2.04(0.67-6.21) | 2.07(0.59-7.22) |
| Anti-N IgG | 0.83(0.47-1.48) | 0.88(0.47-1.65) | 1.05(0.58-1.90) | 0.53(0.44-1.59) |
| Vitamin D | 1.00(0.97-1.03) | 0.99(0.96-1.02) | 0.99(0.96-1.03) | 0.94(0.97-1.03) |
| Vitamin D ≥30 ng/ml | 0.88(0.49-1.56) | 0.90(0.48-1.69) | 0.95(0.62-1.71) | 1.20(0.62-2.39) |
| ABO blood groups | | | | |
| O | 0.65(0.36-1.15) | 0.80(0.43-1.51) | 0.63(0.35-1.15) | 0.69(0.36-1.32) |
| A | 1.36(0.76-2.42) | 1.05(0.56-1.99) | **2.41(1.29-4.49)** | 1.26(0.66-2.41) |
| B | 1.21(0.42-3.49) | 1.57(0.44-5.64) | **0.37(0.14-0.95)** | 0.99(0.31-3.13) |
| AB | 2.16(0.24-18.8) | 1.44(0.16-12.6) | 0.72(0.13-4.04) | NC[2] |

[1] Time since the last vaccine dose or infection. [2] NC= no calculable.

The adjusted analysis of the cellular immune response and associated factors are shown in Table 6. CD8+ T-cell response against Wuhan was significant associated with alcohol consumption (OR= 2.18 95% CI 1.20-4.33), number of SARS-CoV-2 vaccine doses received (OR=1.85 95% CI 1.13-3.03), A blood group (OR=2.61 95% CI 1.37-4.96), and inverse associated with B blood group (OR=0.34 95% CI 0.13-0.89). CD4+ T-cell response against Wuhan was inverse significant associated with physical exercise (OR=0.44 95% CI 0.21-0.99). CD8+ T-cell response against BA.2 was significant associated with age (OR=1.03 95% CI 1.01-1.05), and Anti-N IgM (OR=5.51 95% CI 1.92-25.5), and inverse associated with the time elapsed since the last vaccine dose or infection (OR=0.89 95% CI 0.81-0.99).

**Table 6.** Cellular immune response against Wuhan and BA.2 antigens and associated factors by logistic regression. Total sample. Adjusted odds ratio (aOR) and 95% Confidence Interval (CI). Borriana COVID-19 cohort 2022.

| | CD8+ for BA.2 | CD4+ for BA.2 | CD8+for Wuhan | CD4+for Wuhan |
|---|---|---|---|---|
| Variables | aOR 95% CI | aOR 95% CI | aOR 95% CI | aOR 95% CI |
| Age[1] | **1.03(1.01-1.05)** | 1.01(0.99-1.03) | 1.01(0.99-1.03) | 1.01(0.98-1.03) |
| Male[2] (%) | 1.01(0.53-1.91) | 0.80(0.41-1.56) | 1.13(0.59-2.18) | 0.68(0.26-2.86) |
| Time [3](months) | **0.89(0.81-0.99)** | 0.96(0.87-1.06) | 1.07(0.97-1.19) | 1.0(0.98-1.21) |
| Body mass index [4] (kg) | 0.99(0.94-1.05) | 1.07(0.99-1.15) | 1.02(0.96-1.09) | 1.01(0.94-1.08) |
| Smoking[5] | 0.76(0.38-1.51) | 0.63(0.31-1.30) | 0.82(0.40-1.72) | 0.73(0.34-1.57) |
| Alcohol consumption[6] | 1.18(0.59-2.36) | 1.15(0.55-2.40) | **2.18(1.20-4.33)** | 1.03(0.52-2.42) |
| Physical exercise[7] | 0.66(0.36-1.22) | 0.83(0.44-1.60) | 0.77(0.41-1.41) | **0.44(0.21-0.99)** |
| Chronic Disease[8] | 1.03(0.52-2.07) | 0.90(0.43-1.90) | 0.87(0.42-1.76) | 1.72(0.78-3.79) |

| | | | | |
|---|---|---|---|---|
| Vaccine anti-SARS-CoV-2 | | | | |
| mRNA homologous vaccine[9] | 1.55(0.77-3.11) | 1.04(0.48-2.26) | 0.92(0.44-1.92) | 0.82(0.36-1.87) |
| 3 doses versus[9] 0,1,2 vaccine | 1.24(0.60-2.56) | 0.87(0.39-1.93) | 1.93(0.92-4.05) | 0.74(0.35-1.69) |
| Number vaccine doses[9] | 1.34(0.83-2.17) | 0.99(0.58-1.68) | **1.85(1.13-3.03)** | 1.19(0.68-1.38) |
| Humoral immunity | | | | |
| Anti-S IgG[10] AU/ml | 1.00(0.99-1.00) | 1.00(0.99-1.00) | 1.00(0.99-1.00) | 1.01(0.99-1.00) |
| Anti-N[10] | 1.42(0.74-2.73) | 1.31(0.57-2.21) | 1.06(0.54-2.09) | 0.93(0.46-1.89) |
| Anti-N IgM[10] | **5.51(1.92-25.5)** | 1.01(0.35-2.96) | 2.31(0.71-7.48) | 1.98(0.54-7.28) |
| Anti-N IgG[10] | 1.13(0.59-2.17) | 0.89(0.44-1.80) | 0.92(0.47-1.83) | 0.85(0.42-1.73) |
| Vitamin D[11] | 1.01(0.97-1.04) | 1.01(0.97-1.04) | 1.01(0.97-1.04) | 1.01(0.98-1.05) |
| Vitamin D >29 ng/ml[11] | 1.07(0.56-2.04) | 1.20(0.60-2.40) | 0.95(0.43-1.86) | 1.30(0.64-2.68) |
| ABO blood groups | | | | |
| O[12] | 0.71(0.39-1.24) | 0.83(0.44-1.58) | 0.61(0.33-1.12) | 0.65(0.34-1.26) |
| A[12] | 1.14(0.68-2.25) | 1.01(0.54-1.92) | **2.61(1.37-4.96)** | 1.36(0.70-2.64) |
| B[12] | 1.29(0.43-3.84) | 1.64(0.45-5.89) | **0.34(0.13-0.89)** | 0.92(0.29-2.66) |
| AB[12] | 1.72(0.19-15.8) | 1.37(0.15-12.2) | 0.68(0.11-3.89) | NC[13] |

[1]Adjusted for sex, blood groups, time since the last vaccine dose/infection. [2] Age, blood groups, time since the last vaccine dose/infection. [3] Age, sex, blood groups.[4] Age, sex, blood groups, smoking, alcohol consumption, physical exercise, time since the last vaccine dose/infection.[5] Age, sex, blood groups, alcohol consumption, physical exercise, time since the last vaccine dose/infection.[6] Age, sex, blood groups, physical exercise, time since the last vaccine dose/infection.[7] Age, sex, blood groups, alcohol consumption, time since the last vaccine dose/infection.[8] Age, sex, body mass index, blood groups, alcohol consumption, physical exercise, smoking, time since the last vaccine dose/infection.[9] Age, sex, blood groups, chronic disease, time since the last vaccine dose/infection.[10] Age, sex, blood groups, chronic disease, body mass index, alcohol consumption, physical exercise, smoking, time since the last vaccine dose/infection.[11] Age, sex, blood groups, chronic disease, body mass index, alcohol consumption, physical exercise, smoking, time since the last vaccine dose/infection.[12] Age, sex, time since the last vaccine dose/infection.[13] No calculable.

The Spearman correlation test between Anti-S IgG antibodies and CD4+ and CD8+ T-cell responses against Wuhan and BA.2 antigens are showed in Table 7. Naïve group did not present significant correlations between Anti-S IgG antibodies and CD4+ and CD8 T-cell responses against the two antigens. In contrast, SARS-CoV-2 infected patients had positive correlations between Anti-S IgG antibodies and CD4+ and CD8+ T-cell response against Wuhan and BA.2, and CD4+ T-cell response was significant against Wuhan antigen (rs= 0.198 p=0.006), with marginal signification with the rest of correlations. The patients with sequelae had positive significant correlation between Anti-S IgG and CD8+ T-cell reactive against Wuhan antigen (rs=0.233 p=0.042) and the without sequelae group a significant correlation between Anti-S IgG antibodies and CD4+ T-cell response against Wuhan antigen (rs =0.214 p=0.030). SARS-CoV-2 patients with reinfection or no-reinfection had not significant positive correlations between Anti-S IgG and CD4+ and CD8+ T-cell response against Wuhan and BA.2 antigen. The total of the sample had significant correlation between Anti-S IgG and CD4+ T-cell response against Wuhan antigen (rs = 0.203 p=0.002).

**Table 7.** Spearman correlation test between Ant-S IgG antibodies and CD4+ and CD8+ T-cell response for naïve and SARS-CoV-2 infected patients groups. Spearman correlation coefficient (rs). Borriana COVID-19 cohort 2022.

| Group | CD8+for BA.2 | CD4+ for BA.2 | CD8+ for Wuhan | CD4+ for Wuhan |
|---|---|---|---|---|

| | rs  p-value | rs  p-value | rs  p-value | rs  p-value |
|---|---|---|---|---|
| Naïve group | -0.04   0.812 | 0.014   0.934 | -0.133   0.438 | 0.224   0.188 |
| Infected patients | 0.14   0.055 | 0.134   0.065 | 0.138   0.059 | **0.198   0.006** |
| Patients with sequelae | 0.182   0.112 | 0.116   0.317 | **0.233   0.042** | **0.184   0.108** |
| Patients  without sequelae | 0.143   0.151 | 0.068   0.493 | 0.093   0.348 | **0.214   0.030** |
| Patients reinfection | 0.121   0.293 | 0.142   0.216 | 0.129   0.280 | 0.218   0.055 |
| Patients no reinfection | 0.129   0.232 | 0.163   0.130 | 0.112   0.300 | 0.157   0.144 |
| Total sample | 0.122   0.068 | 0.118   0.078 | 0.103   0.124 | **0.203   0.002** |

Sensitivity analysis

In the sensitivity analysis a total of 175 participants (77.7%) presented positive cellular immune response ≥ 0.10% at least against one antigen, and 50 participants (22.2%) had an immune response inferior or not response. The cellular immune response of CD4+ T cells against Wuhan antigen was 46.8% and against BA.2 antigen was 50.7%, and for CD8+ T- cells against Wuhan 49.3% and against BA.2 47.6%. Comparing naïve and SARS-CoV-2 infection patients, naive group had higher immune response ≥ 0.10% with positive 97% (35/36) versus 74.1% (140/189) and significant difference (p=0.015). In the total, CD4+ T- cell responders were 145 participants (64.4%) and no-responders 80 (35.6%) and CD8+ T- cell responders were147 participants (65.3%) and no-responders 78 (34.7%).

The adjusted sensitivity analysis comparing the cellular immune response of CD4+ and CD8+ T cells among the study groups is showed at Table 8. The naïve group presented higher percentages of immunity response than SARS-CoV-2 infected patients but the differences were not significant. The SARS-CoV-2 patients with sequelae had higher immune response than the no-sequelae group and CD4+ T cells against BA.2 antigens had significant difference (p=0.05). SARS-CoV-2 reinfection groups had not significant immune response CD4+ and CD8+ T cells against Wuhan and BA.2 antigens versus no-reinfection group.

**Table 8.** Sensitivity analysis. Cellular immune response CD4+ and CD8+ T cells positivity  ≥0.10%. Comparisons in naïve group versus SARS-CoV-2 infected patients, SARS-CoV-2 patients with sequelae/ without sequelae, and SARS-CoV-2 reinfection/ no-reinfection groups by logistic regression. Adjusted odds ratios (aOR). 95% Confidence interval (CI). Borriana COVID-19 cohort 2022.

| Groups | CD8+ for BA.2 | CD4+ for BA.2 | CD8+ for Wuhan | CD4+ for Wuhan |
|---|---|---|---|---|
| | % positive | % positive | % positive | % positive |
| Infected patients Cases | 46.6% | 48.7% | 49.1% | 45.5% |
| Naïve  Controls | 52.8% | 61.1% | 50.0% | 53.8% |
| aOR[1]  (96% CI) | 0.74(0.34-1.43) | 0.55(0.24-1.24) | 1.16(0.53-2.55) | 0.73(0.33-1.61) |
| p-value | 0.438 | 0.150 | 0.717 | 0.436 |
| Patients with sequelae Cases | 49.4% | 55.8% | 50.7% | 46.8.% |
| Patients without- sequelae Controls | 45.6% | 41.8% | 47.6% | 44.7% |
| aOR (95% CI) | 0.99(0.52-1.92) | **1.96 (1.00-3.85)** | 1.05(0.55-2.02) | 1.16 (0.60-2.26) |
| p-value | 0.495 | **0.050** | 0.875 | 0.657 |
| Patients  reinfection Cases | 44.9% | 47.4.0% | 43.6% | 43.6% |

| Patients no-reinfection Controls | 51.1% | 50.0% | 51.1% | 50.0% |
|---|---|---|---|---|
| aOR (95% CI) | 0.69 (0.35-1.38) | 0.81 (0.41-1.63) | 0.82(0.42-1.61) | 0.78( (0.40-1.54) |
| p-value | 0.292 | 0.565 | 0.568 | 0.471 |

[1]Adjusted for age, sex, blood groups, body mass index, chronic disease, smoker, alcohol consumption, physical exercise, dose vaccine, time since the last vaccine dose/ infection.

The adjusted analysis of the cellular immune response and associated factors considering as positivity a percentage of ≥ 0.10% are shown in Table 9. Significant associated factors with the immune response against Wuhan antigen were the number of SARS-CoV-2 vaccine doses received (CD8+ T cells), A and B blood groups (CD4+ T cells), and Anti-S IgG (CD4+ T cells). For BA.2 antigen, significant associated factors with the immune response were time since the last vaccine dose or infection, Anti-N IgM (CD8+ T cells), and Anti-S IgG (CD4+ T cells).

**Table 9.** Sensitivity analysis. Cellular immunity response against Wuhan and BA.2 antigens and associated factors by logistic regression. Total sample. Adjusted odds ratio (aOR) and 95% Confidence Interval (CI). Borriana COVID-19 cohort 2022.

| | CD8+ for BA.2 | CD4+ for BA.2 | CD8+ for Wuhan | CD4+ for Wuhan |
|---|---|---|---|---|
| Variables | aOR 95% CI | aOR 95% CI | aOR 95% CI | aOR 95% CI |
| Age[1] | 1.01(0.99-1.03) | 1.01(0.99-1.03) | 0.99(0.97-1.01) | 0.98(0.97-1.01) |
| Male[2] (%) | 1.18(0.67-2.08) | 1.14(0.65-1.99) | 1.06(0.11-1.86) | 1.02(0.59-1.80) |
| Time [3](months) | **0.90(0.83-0.99)** | 1.01(0.93-1.10) | 0.98(0.91-1.07) | 1.06(0.97-1.15) |
| Body mass index [4] (kg) | 0.99(0.94-1.05) | 0.96(0.91-1.01) | 0.99(0.94-1.04) | 0.96(0.91-1.01) |
| Smoking[5] | 0.67(0.35-1.26) | 0.53(0.28-1.01) | 0.83(0.45-1.57) | 0.94(0.50-1.76) |
| Alcohol consumption[6] | 1.06(0.57-1.58) | 1.53(0.82-2.85) | 1.60(0.86-3.00) | 0.90(0.42-1.68) |
| Physical exercise[7] | 0.71(0.41-1.23) | 0.83(0.48-1.43) | 0.89(0.42-1.53) | 0.84(0.49-1.43) |
| Chronic Disease[8] | 1.13(0.62-2.07) | 0.90(0.43-1.90) | 0.87(0.42-1.76) | 1.72(0.78-3.79) |
| Vaccine anti-SARS-CoV-2 | | | | |
| mRNA homologous vaccine[9] | 1.38(0.73-2.61) | 1.33(0.70-2.50) | 1.20(0.64-2.25) | 0.87(0.47-1.64) |
| 3 doses versus[9] :0,1,2 vaccine | 0.96(0.50-1.85) | 0.74(0.38-1.92) | 1.75(0.91-3.38) | 0.87(0.46-1.67) |
| Number vaccine doses[9] | 1.16(0.73-1.83) | 1.11(0.71-1.73) | **1.72(1.05-2.82)** | 1.18(0.76-1.86) |
| Humoral immunity | | | | |
| Anti-S IgG[10] AU/ml | 1.01(0.99-1.01) | **1.01(1.00-1.01)** | 1.00(0.99-1.00) | **1.01(1.0-1.01)** |
| Anti-N[10] | 1.16(0.66-2.06) | 1.63(0.91-2.89) | 0.92(0.52-1.62) | 1.15(0.65-2.04) |
| Anti-N IgM[10] | **2.88(1.11-7.43)** | 2.10(0.84-5.27) | 2.31(0.85-5.31) | 2.27(0.93-5.57) |
| Anti-N IgG[10] | 0.88(0.50-1.57) | 1,24(0.70-2.19) | 0.83(0.47-1.98) | 1.08(0.61-1.91) |
| Vitamin D[11] | 1.02(0.99-1.06) | 0.99(0.96-1.02) | 1.01(0.97-1.04) | 1.01(0.98-1.05) |
| Vitamin D >29 ng/ml[11] | 1.19(0.67-2.12) | 0.73(0.41-1.30) | 0.74(0.42-1.86) | 0.87(0.49-1.55) |
| ABO blood groups | | | | |
| O[12] | 0.76(0.44-1.31) | 0.77(0.45-1.32) | 0.90(0.53-1.54) | 0.76(0.44-1.31) |
| A[12] | 1.62(0.94-2.77) | 1.37(0.81-2.34) | 1.50(0.88-2.56) | **1.72(1.00-2.95)** |
| B[12] | 0.66(0.25-2.76) | 1.64(0.25-1.74) | 0.45(0.16-1.23) | **0.26(0.08-0.82)** |
| AB[12] | 0.43(0.07-2.52) | 1.37(0.32-10.26) | 0.50(0.09-2.83) | 2.66(0.46-15.21) |

[1]Adjusted for sex, blood groups, time last vaccine doses/infection. [2] Age, blood groups, time since the last vaccine dose/infection. [3] Age, sex, blood groups.[4] Age, sex, blood groups, smoking, alcohol consumption, physical exercise, time since the last vaccine dose/infection.[5] Age, sex, blood groups, alcohol consumption, physical exercise, time since the last vaccine dose/infection.[6] Age, sex, blood groups, physical exercise, time since the last vaccine dose/infection.[7] Age, sex, blood groups, alcohol consumption, time since the last vaccine dose/infection.[8] Age, sex, body mass index, blood groups, alcohol consumption, physical exercise, smoking, time since the last vaccine dose/infection.[9] Age, sex, blood groups, chronic disease, time since the last vaccine dose/infection.[10] Age, sex, blood groups, chronic disease, body mass index, alcohol consumption, physical exercise, smoking, time since the last vaccine dose/infection.[11] Age, sex, blood groups, chronic disease, body mass index, alcohol consumption, physical exercise, smoking time since the last vaccine dose/infection.[12] Age, sex, time since the last vaccine dose/infection.

When comparing the sensitivity analysis and the first approach (Table 10), several associated factors maintained signification in both analysis: the time elapsed since the last vaccine dose or infection (CD8+T cell against BA.2 antigen), number of SARS-CoV-2 vaccine doses received (CD8+ T cells against Wuhan antigen), and Anti-N IgM (CD8+ T cells against BA.2 antigen). A and B blood groups were associated but with a change of immunity response from CD8+Tells to CD4+ T cells against Wuhan antigen. However, age, alcohol consumption, and physical exercise lost signification in the sensitivity analysis.

**Table 10.** Associated factors of cellular immunity response CD4+ and CD8+ T cells considering: positivity >0.0% versus positivity ≥ 0.10%. Logistic regression analysis. Adjusted odds ratio (aOR) and 95% confidence interval (CI). Borriana COVID-19 cohort 2022.

| Variables | Positive cellular immune response >0.0% | Positive cellular immune response ≥0.10% |
|---|---|---|
| | aOR     95% CI | aOR 95% CI |
| Age (years) | **1.03    (1.01-1.05)** CD8+ BA.2 | NS[1] |
| Time (months) | **0.89    (0.81-0.99)** CD8+BA.2 | **0.90(0.83-0.99)** CD8+BA.2 |
| Alcohol consumption | **2.18 (1.20-4.33)** CD8+ Wuhan | NS[1] |
| Physical exercise | **0.44(0.21-0.99) )** CD4+Wuhan | NS[1] |
| Number vaccine doses | **1.85(1.13-3.03)** CD8+Wuhan | **1.72(1.05-2.82)** CD8+ Wuhan |
| Anti-S IgG AU/ml | NS[1] | **1.01(1.00-1.01**) CD4+BA.2; CD4+Wuhan |
| Anti-N IgM | **5.51(1.92-25.5**) CD8+BA.2 | **2.88(1.11-7.43**) CD8+BA.2 |
| A blood group | **2.41(1.29-4.49)** CD8+Wuhan | **1.72(1.00-2.95)** CD4+Wuhan |
| B blood group | **0.37(0.14-0.95)** CD8+Wuhan | **0.26(0.08-0.82)** CD4+Wuhan |

[1] NS = No significant.

## 4. Discussion

In our cohort with high percentage of three doses mRNA vaccinated, an elevate percentage of participants were positive CD4+ and CD8+ T-cell immune response against Wuhan and BA.2 antigens. The naïve group had a cellular immune response comparable to SARS-CoV-2 infected patients. SARS-CoV-2 patients with sequelae had a more significant immune response against Wuhan and BA.2 antigens than the patient without sequelae group. On the other hand, significant correlations between Ant-S IgG and immune response CD4+ and CD8+ T cells against Wuhan and BA.2 antigens were found in the SARS-CoV-2 infected patients.

Considering associated factors with T-cell response, highlighting positive associated factors such number of SARS-CoV-2 vaccine doses received (CD8+ against Wuhan antigen), Ant-N IgM (CD8+ against BA.2 antigen), and A-blood group (CD8+ against Wuhan antigen). In addition, inverse associated factors with immune response were the time elapsed since the last vaccine dose or

infection, and B blood group (CD8+ against Wuhan antigen).    High age, alcohol consumption and physical exercise were associated with the immune response against BA.2 and Wuhan antigens, but the sensitivity analysis had not found these associations.

In our cohort, the percentage of CD4+ and CD8+ T-cell responders was higher than that observed in other studies at population level or in healthcare workers with cross-sessional [30,31], or cohort designs [13,32–37]. Although several tests to measure immune response were used with different SARS-CoV-2 variants, demographic differences, and the follow-ups were variable but 6 months at minimum.

Detection of CD4+ and CD8+ T cells after the first dose of mRNA SARS-CoV-2 vaccines have been found [19,38–41] with a duration of   6 months and more [42] and our results after 11 months of the last vaccine dose in the naïve group are in this line. In the SARS-CoV-2 infection patients, CD4+ and CD8+ T-cell response have been indicated after 8 months of the onset of the disease with CD4+ increased versus CD8+[43]. On the other hand, decline of T-cell response had been described [44,45], and CD4+ presented more decline than CD8+ [46]. In addition, SARS-CoV-2-reactive CD4+ T cells had detected in 40%-60% no exposed individuals possible associated with circulating coronavirus [47].

Presence of CD4+ and CD8+ T cells may indicate a protection against COVID-19, considering that SARS-CoV-2 infected patients with SARS-CoV-2 vaccine have the named hybrid immunity (HI) (infection plus vaccination), and consequence less severity and hospitalizations in reinfections [48], and HI supposes a more robust cellular immunity and increased of SARS-C neutralizing antibodies [49–51]. Primorac and co-authors [52] found less SARS-CoV-2 infection or reinfection with high level of cellular immune response after vaccination and/or previous SARS-CoV-2 infection. Zens and co-autors [13]   in a cohort study with 141 participants found that IFN-gamma-producing S-reactive T cells presented significantly less risk of SARS-CoV-2 infection or reinfection. In contrast, T-cell response did not reduce breakthrough risk of SARS-CoV-2 infection in an open-label trail in Austria [53], in a Danish cohort study [35], and in mRNA vaccinated nursing home residents in Spain [54].

Cellular immune response against SARS-CoV-2 may have differences in the populations of T cells considering quantity, localization and functionality with variations depending of epidemiological, virological, and immunological situation [43,55]. Then, a SARS-CoV-2 protection may depend of variants, time since the last infection, or vaccine doses, or inoculum size. In addition, some virus variant as Omicron does not produce T-cell boost [56], and profile complementary determining regions for HI and not HI have been indicated   [57].

However, how the T cells protect against severe SARS-CoV-2 is not well demonstrated, and Kent and co-authors [12] have indicated no association between getting a SARS-CoV-2 infection and either T-cell responses measured in blood, considering that T-cell response has not been measured in mucosal or tissues and there is not a standardized T-cell assay for comparison with different studies. In addition, there are several tests to determine T-cell response including: flow cytometry immunoassay for intracellular cytokine staining [24], activation induced marker assay [35], immunosorbent spot (FluroSpot) assay [58] and enzyme-linked immunospot (ELISpot) and cytokine secretion [59,60]. These tests are difficult to implement at the population level considering the high labor they need and the low performance [13], and other tests such as interferon-$\gamma$ release assay (QuantiFERON® SARS-CoV-2 Test) [61] are being used, although the sensitivity is lower [62,63].

The cellular immune response had equivalent levels in the naïve group and SARS-CCoV-2 infected patients is in line with Camacho and co-authors [30] that found no difference between vaccinated infected cases and vaccinated naïve participants in a cross-sectional study in a general population of Valencia Community, with Paniskaki et co-authors   [64] in a cohort of vaccinated naive and vaccinated SARS-CoV-2 patients in Germany, and   with Gatti and co-authors in a comparison between SARS-CoV-2 convalescent and naïve vaccinated in Milan after 2 years of infection [65]. In addition, a study of nursing home [66] naïve residents presented cellular immune response comparable with SARS-CoV-2 patients after a third dose of mRNA of Comirnaty vaccine. In naïve participants, using interferon-$\gamma$ release assay to the cellular immune determination after mRNA SARS-CoV-2 vaccine, T-cell response had found [67]. In addition, De Marcos and co-authors

[37] found no difference in cellular immune response in a cohort of vaccinated health workers naïve or SARS-CoV-19 infected to Omicron variant.   In Brasil, Azamor and co-authors [68] found that after 120 days of the second vaccination with ChAadOx1 nCoV-19 the percentages of CD4+ and CD8+T cells were higher in a no SARS-CoV-2 group compared with a SARS-CoV-2 group. Our results of cellular immune response in the naïve group and the SARS-CoV-2 infection patients are in this line. In contrast, Moore and co-authors [34] in a cohort of 684 health care workers in England found higher T-cell immune response in vaccinated with a prior SARS-CoV-2 infection than naïve vaccinated after 6 months of the vaccination, and suggesting that with the time the two immune responses from infection and/or vaccination will be similar. However, the immune response to the new variants of SARS-CoV-2 may present differently with respect to protection in the infected and naïve groups [69].

In our results, SARS-CoV-2 patients with sequelae had more T-cell immune response CD4+ against BA.2 and Wuhan antigens, and CD8+ T cells against Wuhan antigen than SARS-CoV-2 patients without sequelae. This may suggest a more recent reinfection BA.2, variant predominant at our zone in 2022 year, and persistent effect of Wuhan variant. Some authors have found that patients with long COVID-19 present alteration of CD4+ and CD8+ T-cell populations that could be associated with viral persistence [70]. In long COVID-19 patients had been observed an increase of CD4+ and CD8+ secreting IFN-gamma [71], and Cruz and co-authors [72] found increased of both CD4+ and CD8+ T cells in long COVID-19 patients with lung sequelae. In patients with the post COVID-19 syndrome, Acosta Ampudia and co-authors [73] found increased levels CD8+ effector T cells, CD4+ effector memory T cells after 11 months of following. Paniskaki and co-authors [74] in SARS-CoV-2 patients with post sequelae found an intense SARS-CoV-2 reactive CD8+ T cell response.

However, other authors found lower cellular immune response in severe SARS-CoV-2 patients such as in Italy [75]. In addition, Wu and co-authors [76] found similar levels of CD4+ and CD8+ T cells in SARS-CoV-2 patients with or without pulmonary sequelae, although patients with pulmonary sequelae presented intense disparity immunity with increased proportion of natural killer and low percentage of B cells. In contrast, others authors found increment of CD8+ T-cell percentages in post-acute of SARS-CoV-2 patients with sequelae [77], or relating to the severity of the disease [32,33]. In addition, an increase of a marker of T-cell exhaustion (sTim-3) in a Norwegian cohort of SARS-CoV-2 patients hospitalized and follow-up 3 months after discharge could be associated with long-term outcomes after a severe disease [78].Then, an elevated heterogeneity in the immune response has been indicated considering the different clinical outcomes and highlight in the heterogeneity level of CD4+ T-cell response [4,79].

In relation to the associated factors with the cellular immune response estimated in our study, the number of SARS-CoV-2 vaccine doses received was associated with an increase of T-cell response such as has been indicated in previous studies [9]. The number SARS-CoV-2 mRNA vaccine doses increased the immune response, measured by flow cytometry, in a cohort in the United States [80], and it was associated with CD4+T cell response in a cohort of Norwegian senior [81]. Age was associated with a positive T-cell response in line with the study of Costa and co-authors [36] in a cohort of healthcare workers using QuantiFERON® SARS-CoV-2 assay in Italy. However, in nursing-home residents the rate and frequency of detectable SARS-CoV-2 IFN-$\gamma$ T-cell responses after vaccination was lower than in controls in Spain [54], and in England, residents of long-term care facilities presented lower T- cell response with higher age [82]. In Greece, a cohort of healthcare workers, female had a high T-cell response measured by QuantiFERON® SARS-CoV-2 assay [83]. In Ireland, Townsend L and co-authors [84], using flow cytometry in a cohort of 71 COVID-19 patients and   40 no infected controls, reported that activated CD4+ and CD8+ T-cell response increased with age, and sex was not associated. However, in Denmark Dietz LL and co-authors [35] by activation induced marker assay, indicated a hypo cellular immune response in males, and age older than 75 years but without significant differences in a cohort of 655 older subjects and mRNA-1273 vaccines increase T-cell response. In Brazil, Fernandes and co-authors [45] using a human IFN-$\gamma$ ELISpot assay found heterogeneous T-cell responsiveness, decreased in male, older patients and no-hospitalized patients   in a cohort of convalescents   and no-exposed controls. In another cohort study using enzyme-linked immune-spot assay [85] no association with age or sex was found. In Japan, Tani and

co-authors [31] found by a standardized   ELISpot interferon–gamma release assay that after booster mRNA SARS-CoV-2 vaccine, an increase of T-cell response in participants age ≤40 years and those with adverse reactions at second or third vaccine dose.

In according with Costa and co-authors [36], who found that no-O blood groups were associated with increase T-cell response, the A blood group had higher significant CD8+ T-cell response, but the B blood group had a significant inverse response. In addition, Gil-Manso and co-authors [86] found that O blood group individuals presented significantly lower frequencies of specific CD4+ T-cell responses against non O blood group individuals. However, no associations between blood-groups and cellular immune response have been found in other studies [31].

Time elapsed since the last vaccine dose or the infection was inverse associated with a low cellular immune response in line with a study of SARS-CoV-2 convalescents and controls [45], in a cohort of SARS-CoV-2 patients [44] and cohorts of healthcare workers in Italy [36], and in Bulgaria [87]. Alcohol consumption was associated with CD8+ T cell response. In contrast, Tani and co-authors [31] had no found this association in a cohort of mRNA vaccinated. In the other hand, higher lymphocyte counts in people who had alcohol consumption have been found [88]. Smoking was not associated with T-cell response in line with Tani and co-authors [31]. However, Costa and co-authors [36] found that current smoking increased T-cell response. Physical exercise showed and inverse association with CD4+ T-cell response against Wuhan antigen, suggesting low protection against SARS-CoV-2. This contrasts with an experimental clinical trial in Arizona, where exercise after SARS-CoV-2 vaccination was associated with robustly mobilized SARS-CoV-2-specific T cells, but only in SARS-CoV-2 patients and no-infected participants presented reduced T-cell response [89]. In addition, Barni and co-author [90] have indicated in a review study that exercise increased amount of CD4+, IL-6 and leukocytes, in patients who performed physical exercise compared to the control group.   However, the effect of exercise as increase susceptibility of infection is a debated subject [91]. In relation to BMI, no increase of T-cell response had found in line with other studies [81,92]. Chronic disease prevalence was not associated with   a T-cell response as it has been found in some studies [81] in contrast with other studies where chronic diseases were associated with T-cell response [11,93]. Vitamin D levels had no effect on T-cell response although their actions as immune regulator [94]. Association of Anti-S IgG with T-cell response has been indicated [43]. However, no correlation has been found in in a cohort of SARS-CoV-2 patients in Cambodia with a follow-up of 9 months [95]. No significant difference of Anti-S IgG levels between the groups was found, unless the SARS-CoV-2 no-reinfection group had higher Anti-S IgG levels than the SARS-CoV-2 reinfection group, suggesting immunity protection in the first group [96,97]. Of note, Anti-N IgM was associated with CD8+ T -cell response against BA.2 antigen in concordance with the SARS-CoV-2 variant more frequent in our zone during the 2022 year.

This study has several strengths, including: First, the follow-up of this cohort could offer more knowledge of the dynamic of the SARS-CoV-2 pandemic. Second, the participation rate of this cohort was more than 60%. Third, the nested case-control design in a cohort is useful to measure variables which required complex tests [98]. Fourth, the measurement of variables was made before the nested case-control was carried out that reduces information bias. Fifth, a random sampling of the cohort participants was obtained to performer the nested case-control study. Sixth, the test to determine cellular immune response was the flow cytometry for intracellular cytokine staining assay that has high sensitivity comparing with other tests [21]. Seventh, the results have been adjusted for potential confounding by multivariable logistic regression models.

Our study presents limitations including: First, cellular immune memory has multiple components and we are studied only SARS-CoV-2-S-reactive interferon-γ-producing CD4+ and CD8+ T cells as a measure of cellular immune response [99]. Second, the used flow cytometry for intracellular cytokine staining assay is a test not standardized yet [21]. Third, just two SARS-CoV-2 variants, Wuhan and BA.2, were included in the study. Fourth, only T-cell interferon-γ production functionality was studied [26,100]. Fifth, our sample size is more or less comparable with many studies of SARS-CoV-2 cellular immune response but could be considered with small power when comparisons inside groups have made. Sixth, a measure of SARS-CoV-2 exposure could be

improving our results [9]. Seventh, information on sequelae was obtained from the participants themselves. Eighth, some SARS-CoV-2 reinfections may be misclassified considered the reinfection definition of the European Center Disease and Prevention [28] Ninth, in the cohort the COVID-19 disease was mild with few severe cases and hospitalizations, and it may be no generalized for the SARS-CoV-2 pandemic. Tenth, COVID-19 disease is a new and some potential factors may be not included in this study. Eleventh, although the results are adjusted some residual bias may continue. Twelfth, no SARS-CoV-2 variants of infected patients were obtained.

The analysis of sensitivity has produced a more detail approximation to the immune response of our cohort. The results of cellular immune response comparing naïve group and SARS-CoV-2 infected patients were similar, and significant differences between SARS-CoV-2 patients with sequelae versus patients without sequelae were maintained, and the consistence of four associated factors suggests the importance of these factors in the dynamic of cellular immunity.

## 5. Conclusions

In a sample of our cohort, cellular immune responses were elevated and comparable between naïve and SARS-CoV-2 groups, but differences in these responses were found in SARS-CoV-2 patients with sequelae versus patients without sequelae.  In the two analysis, several factors were associated with the T-cell response including number of SARS-CoV-2 vaccine doses received, time elapsed since last vaccine dose or infection, A and B blood groups, and presence of Anti-N IgM. In addition, Anti-S IgG was correlated with the cellular immune response. The results of the sensitivity analysis were consistent with the first analysis. The study adds information in cellular immune response that could be useful to the surveillance of the SARS-CoV-2 pandemic, and these findings contribute valuable insights into the nuance immune landscape shaped by SARS-CoV-2 infection and vaccination.

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**Informed Consent Statement:** All participants or the parents of minors provided written informed consent to be included in this study.

**Data Availability Statement:** Data from this study can be consulted if the authors are requested.

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