Monitoring humoral responses against three SARS-CoV-2 vaccines in a university population from Chihuahua, Mexico

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Abstract

Introduction: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has spread worldwide since 2019. Survey of the antibodies against SARS-CoV-2 is one of the most important measures of immunity since it can give an idea on the effectiveness of administered vaccines and the serologic status of individuals. We determined the concentrations of blood IgM and IgG against three SARS-CoV-2 proteins in vaccinated teachers and students among a university population from Chihuahua, Mexico.

Methodology: Humoral response surveillance against the 3C-like proteinase (3CLpro), nuclear protein (NP), and receptor binding domain (RBD) of SARS-CoV-2 was carried out. A total of 239 samples were analyzed: 67 from teachers who were vaccinated with CanSino and 172 from students (27.9% were vaccinated with AstraZeneca, 32.6% with Sinovac, 24.4% with Pfizer-BioNTech, 15.1% with other vaccines).

Results: Significant differences in the levels of IgG were observed between serum from individuals prior to vaccination (preimmunization serum) and from those that were vaccinated with CanSino. However, samples from asymptomatic individuals did not show differences between the preimmunization and post-immunization serum. The three vaccinated groups (AstraZeneca, Pfizer and Sinovac) did not show significant differences in anti-RBD IgG antibody titers compared to the positive control group, except for a Pfizer non-COVID-19 subgroup where the level of antibodies in the Pfizer group was 1.7 times higher. Neither vaccine group showed significant differences between those individuals who previously had COVID-19 and uninfected individuals.

Conclusions: These results provide a picture of the situation at the time when in-person classes resumed.

Key words: SARS-CoV-2; antibodies; vaccines; teachers; students.

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Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19) and has spread worldwide since 2019. The limited immunity of the population against this new coronavirus caused, until May 2023, 765,903,278 confirmed cases of COVID-19 and 6,927,378 deaths [1,2]. In the face of this pandemic, diverse protective activities were invoked throughout the world, including academic responses adapted in various ways, via both at home and online assignments and classes, until effective vaccines against COVID-19 could be developed and deployed [3,4].

Protective immunity against SARS-CoV-2 is based on both humoral and cellular immune responses. The immune response against the virus depends to a large extent on antigenic specificity, especially to antigens such as the N-terminal domain (NTD) and the receptor-binding domain (RBD) of the spike (S) glycoprotein [5].

As of December 2, 2022, 50 different COVID-19 vaccines have been approved in at least one country [6]. Most of the vaccines were based mainly on the viral S glycoprotein, which was administered by various methodologies such as non-replicating adenoviruses, nucleic acid vectors, mRNAs, recombinant proteins,

and inactivated viruses that, in addition to the S protein, also include the entire viral structure [4].

Among the main vaccines applied worldwide were the spike mRNA vaccines BNT162b2 (Pfizer-BioNTech, Michigan USA) and mRNA-1273 (Moderna, Marlborough, Massachusetts); as well as Ad26.COV2-S (Johnson & Johnson's Janssen Division, Beerse, Belgium) consisting of an adenovirus vector encoding a stabilized spike protein; Ad5-nCOV (CanSino Biologics Inc. and the Beijing Institute of Biotechnology, Tianjin, PRC), an adenoviral vector fused with full-length spike protein; ChAdOX1-nCoV (AstraZeneca/University of Oxford, London, UK), an adenovirus vector encoding full-length spike protein; and Sinovac (Sinovac Biotech, Hong Kong S.A.R., PRC), made from the entire inactivated virus [7,8].

Evidence from clinical trials and observational studies overwhelmingly support the safety and efficacy/effectiveness of numerous COVID-19 vaccines to reduce the incidence and severity of SARS-CoV-2 [9]. Specifically, antibodies to SARS-CoV-2 produced after vaccination have been shown to protect against severe disease in a range of 62 to 90% depending on the dosage, in addition to a wide range of effectiveness (50 to 100%) against infection [9]. For example, it was found that 100% of individuals vaccinated with the mRNA vaccines BNT162b2 and mRNA-1273 tested positive for IgGs against the S1 region, mainly for anti-RBD and 97% of individuals vaccinated with the vector-based vaccine ChAdOx1 developed S1-specfic IgG antibodies [10]. The Ad5-nCOV vaccine showed seroconversion rates at 96% for antibodies against the RBD [11].

Due to the data obtained from clinical trials of the different vaccines together with the need to continue and return to productive activities, the COVID-19 pandemic has forced health authorities around the world to deploy different vaccination plans. In Mexico, it was decided to start vaccination in December 2020 in the population considered most at risk, including healthcare personnel, with the BNT162b2 vaccine from Pfizer-BioNTech [12,13]. The educational personnel were exclusively administered the Ad5-nCoV CanSinoBio vaccine, which was authorized on February 8, 2021, and administered during April and May 2021 [14,15].

After vaccination of the school teaching and administrative staff, the Mexican Public Education Secretariat (SEP) schools were urged to reopen starting January 2022 for in-person activities in basic education throughout the country [16]. In contrast, higher education hybrid academic activities were restarted in the second half of 2021. At the local Autonomous University (UACH), in the city of Chihuahua, preference was given to participants in courses that would require practical settings, e.g., workshops and laboratories. To enable this, all UACH staff (administrative and teaching) were vaccinated with the CanSinoBio vaccine in May 2021 [17–19].

Knowing the duration of immunity produced by SARS-CoV-2 vaccines is essential to guarantee the health of individuals during the return to face-to-face activities. Therefore, it is important to assess how long antibodies persist after natural infection and after immunization, as well as their protective capacity [20]. In a study carried out at the University of Salamanca, a general Ig seroprevalence of 8.25% was found in teachers and students before vaccination [21]. In England, the seroprevalence in university students was 17.8% prior to vaccination and during return to school [22]. On the other hand, in students and staff of secondary schools, a seropositivity of 13.1% and 13.3%, respectively, was found before vaccination [23]. During the first wave of COVID-19 in Mexico, the estimated national seroprevalence of antibodies against SARS-CoV2 was 3.5% in February 2020 [24] and 24.9% in November 2020, being lower in adults over 60 years of age and higher in urban areas [25]. In clinical trials, a group of childcare workers immunized with a single dose of the CanSinoBio (Adv5-nCoV) COVID-19 vaccine had an estimated vaccine effectiveness of 20% against disease, 76% against hospitalization, and 94% against death [26]. In another clinical study where the percentage of neutralizing antibodies against SARS-CoV-2 was explored, it was found that the Pfizer/BioNTech, Moderna, and CanSinoBio vaccines showed the highest percentages of neutralization with means of 97.23%, 97.61%, and 97.23%. respectively [27].

The determination of antibodies against SARS-CoV-2 is of the utmost importance since it can give an idea of both the effectiveness of the vaccines and the duration of immunity against the virus. Therefore, our objective was to determine the concentrations of IgM and IgG against the RBD of the spike glycoprotein, the phosphoprotein nucleocapsid (N) and the M protein in UACH teachers and post-vaccination students administered with CanSinoBio, AstraZeneca, Pfizer, and Sinovac vaccines.

Methodology

Ethics statement

This study was approved by the Facultad de Medicina y Ciencias Biomedicas Program Institutional

Review Board (CI-015-20) and the Ethics Review Board from Secretaría de Salud del Estado de Chihuahua (SI-002-2020). Informed consent was obtained from all enrolled patients and healthcare workers.

Patients/donors

Teachers, new students from the UACH 2021-22 cohort (fall semester), and all enrolled Biomedical Engineering students (regardless of semester) were eligible for characterization of humoral immune response induced by vaccination against SARS-CoV-2. Sixty-seven samples from teachers were included in the study; all of them were vaccinated with CanSino. One hundred and seventy-seven samples from healthy students were characterized. Among these, 172 samples were from students who received either AstraZeneca, Sinovac, Pfizer-BioNTech, CanSinoBio, or Moderna vaccines. A total of 103 students reported not having COVID-19 prior to vaccination, while 69 reported COVID-19 infection before being vaccinated. Of the participants, 27.9% were immunized with AstraZeneca, 32.6% with Sinovac, 24.4% with Pfizer-BioNTech, and 15.1% with other vaccines (CanSinoBio, Moderna, and Johnson & Johnson) (Table 1).

Blood samples were collected in tubes without anticoagulant and centrifuged to separate sera. Convenience sampling was carried out during August-December 2021. Paired whole blood was collected in sodium heparin-coated vacutainers and kept on gentle agitation until processing. All blood was processed on the day of collection. Plasma samples from 35 patients infected with SARS-CoV-2 and confirmed by real-time polymerase chain reaction (RT-PCR), were used as positive controls. Healthy donor (HD) plasma was obtained from 16 persons without any vaccine, during the months of June to August 2021.

Table 1. Cohort baseline characteristics.

Characteristics	Subjects
General information	**n = 177**
Gender (male/female)	81/96
Mean age ± SD	20 ± 3
COVID-19 infection	**n = 172**
No COVID-19 prior to vaccination	103
Had COVID-19 prior to vaccination	69
Vaccines	
AstraZeneca	27.9% (48/172)
No COVID-19 before vaccination	29/48
Had COVID-19 before vaccination	19/48
Sinovac	32.6% (56/172)
No COVID-19 before vaccination	32/56
Had COVID-19 before vaccination	24/56
Pfizer-BioNTech	24.4% (42/172)
No COVID-19 before vaccination	28/42
Had COVID-19 before vaccination	14/42
other vaccine	15.1% (26/172)
No COVID-19 before vaccination	14/26
Had COVID-19 before vaccination	12/26

COVID-19: coronavirus disease 2019.

Isolation of patient plasma

Plasma samples were collected after centrifugation of whole blood at 400 g for 10 min at room temperature (RT) without brake. The undiluted serum was then transferred to 15 mL polypropylene conical tubes, and aliquoted and stored at -80 °C for subsequent analysis. Plasma obtained from patients was heat-inactivated (56 °C, 30 min).

Recombinant proteins

The SARS-CoV2 antigens used were the RBD of the spike glycoprotein, N protein, and 3C-like proteinase (3CLpro). The N protein was expressed and purified using the pET-28a+ *E. coli* vector (Novagen, Pretoria, South Africa) with optimized codons for residues 173-591 (sequence ID: QIC50514.1) inserted between the *Nhe I* and *Not I* sites. A 1L culture of the *E. coli*, DE3 strain containing this vector was used to produce 174 mg of Ni++ affinity-purified protein using methods described in [23] with final formulation in phosphate buffered saline (PBS) at 3 mg/mL. Chain A, 3CLpro (GenPep Sequence ID: 6XA4_A) was produced in a manner similar to the N protein. The RBD protein sequence ID: QJD23474.1 residues 172-395 fused to ASHHHHHHAS and followed by EPEA (Glu, Pro, Glu, Ala) and was expressed in transiently transfected Chinese hamster ovary (CHO-S) cells using a previously described CET-HS mammalian expression vector (Cat UC0E01, EMD Millipore Corporation, Temecula, CA) [23,24] with TransIT-VirusGEN® (Merck, Rahway, New Jersey) transfection reagent according to the manufacturer's protocol (Mirus, WI, USA). Culture supernatant (220 mL) was loaded onto 2 connected 1 mL CaptureSelect™ C-tag XL pre-packed columns (Thermo Scientific, Waltham, MA) at 1 mL/minute, then washed with PBS at 0.5 mL/minute for 1 h, and eluted with 2M $MgCl_2$ in 20 mM Tris-HCl (pH 7.0) at 0.5 mL/minute. The fractions were analyzed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and those with RBD were pooled and dialyzed into PBS with Ca/Mg to yield 10 mg of recombinant protein.

Enzyme linked immunosorbent assay (ELISA)

Clear flat-bottom Nunc MaxiSorp™ 96-well plates (442404, ThermoFisher, Waltham, MA) were coated with 50 mL of 2 μg/mL antigen in 50 mM

carbonate/bicarbonate buffer, pH 9.6 overnight at 4 °C, washed in PBS with 0.05% Tween 20, blocked with 65 mL tris buffered saline (TBS) (SuperBlock™, ThermoFisher, Waltham, MA) for 1 h at 37 °C, and then patted dry. Thawed plasma samples (50–100 mL) were spun at 2,500 rpm in a refrigerated microfuge for 10 min at 4 °C before dilution in TBS. For the N or 3CLpro-point assay, dilutions of 1:250 and 1:500 were used. For the RBD spike antigen 2-point assay, dilutions of 1:100 and 1:250 were used. Samples (50 mL) were applied to the antigen-coated plates, covered with plate sealer, and incubated at 4 °C overnight. On the following day, the plates were washed 3× in PBS with 0.05% Tween 20. Plate-bound IgG was detected with 50 mL of peroxidase-conjugated donkey anti-human IgG Fc specific reagent (709-035-098; Jackson ImmunoResearch, West Grove, PA) at a 1:25,000 dilution or peroxidase-conjugated goat anti-human IgM Fc 5μ specific reagent (109-036-129; Jackson ImmunoResearch, West Grove, PA) used at 1:5,000 in TBS for the N protein assay and a 1:10,000 dilution in TBS for the RBD assay. The plates were covered with plate sealer, incubated at 37 °C in a 5% CO_2 humidified incubator for 1 hour. Plates were then washed in PBS with 0.05% Tween 20, developed with 50 mL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Life Technologies, Waltham, MA) for ≤ 5 min, stopped with 50 mL of 1 N HCl, then read at absorbance 450 nm on a MultiSkan FC instrument (Thermo Scientific, Waltham, MA). All manipulations with open plates were done in a SterilGARD® III laminar flow hood (Baker, Waltham, MA). For serial dilution titrations, the same overall process was used. To choose the threshold value, the standard deviation of the reading absorbance at 450 nm of the negative controls and pre-immune sera was assayed, determining that the threshold value was at least three times the standard deviation for each protein.

Data processing and analysis

Calculations were performed using Microsoft Excel 365. Fitting curves to find the absolute EC_{50} were determined by plotting the $\log_{10}$ of the dilution factor values (x-axis) required to obtain OD 450 nm values (y-axis). Analyses were performed using GraphPad Prism 9 software and the figures were drawn using Prism 9 (GraphPad Software, Boston, Massachusetts USA). The Shapiro-Wilk test was used to test the normality of a data set. The Krustal-Wallis method was used to evaluate the effect of COVID-19 infection prior to immunization, gender, and the different vaccines on the antibody titers against the three SARS-CoV-2 proteins. $p < 0.05$ was taken as the level of significance.

Table 2. Teachers' cohort baseline characteristics.

Characteristics	Subjects
General information	**n = 67**
Gender (Male/Female)	40/27
Age range	20-49
Vaccinated CanSino participants	**n = 67**
No COVID-19 before vaccination	52
Had COVID-19 before vaccination	15

COVID-19: coronavirus disease 2019.

Results

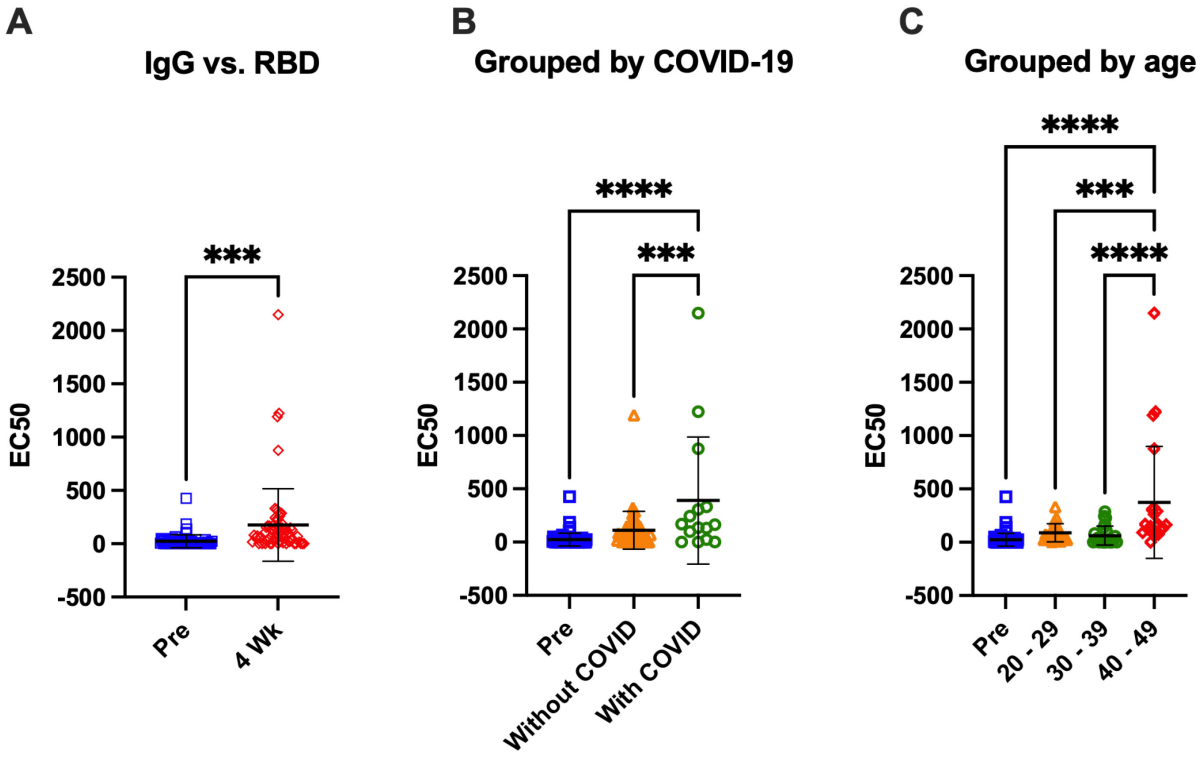
Serum antibody levels among teachers vaccinated with Ad5-nCOV (CanSinoBio)

A total of 67 blood samples were collected from university teachers vaccinated with a single dose of CanSinoBio vaccine. All of them had their blood sample taken before being vaccinated and four weeks after receiving the vaccine. It should be noted that the range of donors' age was between 20 to 49 years (Table 2). At the time the samples were taken, participants or teachers who were ≥ 50 years and received the Pfizer-BioNTech vaccine and were not considered in this group/study. Significant differences in the levels of IgG antibodies were observed between pre-vaccination samples and the first and only vaccine administration. However, when analyzing the samples and separating by record of COVID-19 infection, people who had not reported to have had the disease, did not show differences between the pre-vaccination and post-vaccination serum. In contrast, in people who had reported COVID-19 infection, there were significant differences when compared to the pre-pandemic control and people with no reported infection. The only group that presented significant differences after vaccination were the 40-49 years old teachers (Figure 1).

RBD protein

Regarding IgM antibodies against the RBD protein, the titers did not show significant differences between the groups of individuals previously infected, individuals who did not have COVID-19, and the negative and positive controls. In the case of IgG antibodies, there was a significant increase in antibody concentration in the serum of those who were vaccinated, who previously suffered from COVID-19, and those who did not have COVID-19, when compared to the value of the negative control sera (Figure 2, with $p < 0.0001$ in both cases). The average value of IgG-RBD antibodies was 0.94 and 1.0 in those vaccinated against COVID-19 and without COVID-19

Figure 1. Titer of IgG isotype antibodies against the receptor binding domain (RBD) protein in a population of university teachers with CanSinoBio vaccination.



A: Monitoring of the humoral immune response (RIU) before and four weeks after vaccination, paired t test $p < 0.05$; B: groups separated according to whether or not they had reported COVID-19 and its monitoring by the RIU after 30 days, Tukey multiple comparison test, $p < 0.05$; C: groups separated by age and their monitoring 30 days after being vaccinated, Tukey multiple comparison test, $p < 0.05$.

infection, respectively; four times higher than the average value of the negative control. In 72% of those vaccinated without previous COVID-19 infection, this value exceeded the threshold value of 0.5 (developed anti-RBD IgG antibodies), while in those vaccinated and with previous COVID-19 infection, 71% exceeded the threshold value of 0.5. However, no significant differences were found in the values between those who were positive for a previous SARS-CoV-2 infection and those who were not ($p > 0.05$); so, it can be determined that the anti-RBD antibody response to vaccination was independent of having suffered or not from COVID-19 (Figure 2).

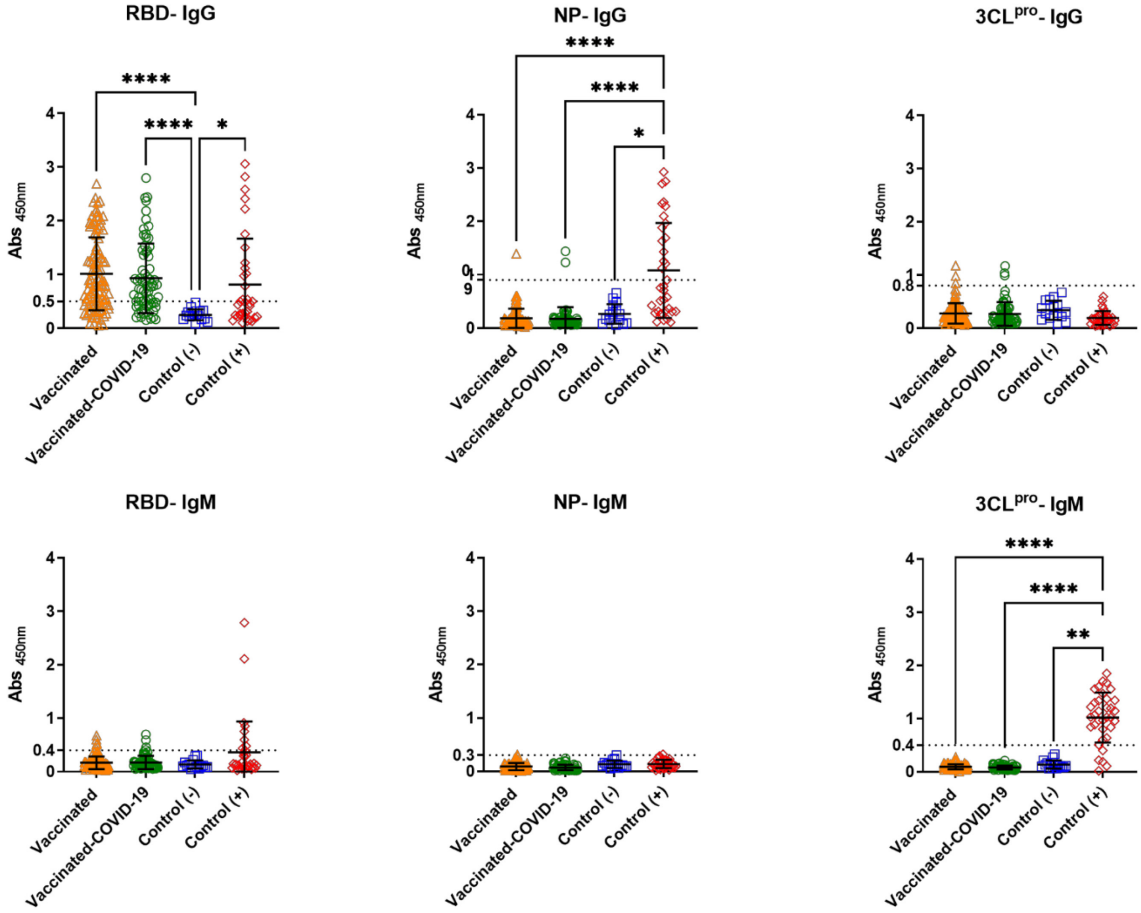
N protein (N)

When evaluating IgM antibodies against N protein, the titers did not show differences between the groups evaluated (Figure 2). On the other hand, in the case of anti-N IgG antibodies, the behavior was opposite to that previously described (IgG-RBD), with a significant difference in the values of those vaccinated with COVID-19 infection and those who did not have COVID-19 compared with the positive control ($p < 0.0001$ in both cases). The mean was six times lower than the mean of the positive control and two times lower than that of the negative control, showing that there was no increase regardless of COVID-19 status. Only two vaccinated individuals, one with COVID-19 and one without infection exceeded the threshold value of 0.9, indicating that they developed IgG antibodies against the N protein. It should be noted that one of the subjects suffered from COVID-19 on two separate occasions while another reported a natural infection with SARS-CoV-2, which may explain the anti-N IgG levels being above the threshold. However, a third anti-N IgG positive individual did not indicate that he developed a previous infection, so his IgG levels against the N protein may be due to a recent infection that progressed without apparent symptoms (Figure 2).

3C-like proteinase (3CLpro)

Anti-3CLpro IgM had an average value of 0.08 and 0.10 in those vaccinated with COVID-19 and without COVID-19, respectively (Figure 2). The mean difference between them being 13 times less in the COVID-19 vaccinated group, compared to that of the positive control group; whereas in those vaccinated and without COVID-19, it was less than 11 times. On the

Figure 2. Values from anti-SARS-CoV-2 antibodies.



Anti-receptor binding domain (RBD), anti-nuclear protein (NP) and anti-3C-like proteinase (3CLpro) IgM and IgG levels in patients who had COVID-19 and who did not have COVID-19 before vaccination. The dotted line indicates the threshold value of the requirement. Kruskal-Wallis analysis is presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

other hand, the values of IgG-Mpro, did not show a significant difference between the sera of those vaccinated with COVID-19 and the controls ($p > 0.05$) as in the case of the sera from those who were vaccinated and without COVID-19. It is worth mentioning that five individuals exceeded the threshold for anti-3CLpro IgG antibodies, of which three reported having had COVID-19 at least once while the other two did not indicate having suffered from the disease; however, it is possible that their levels above the threshold may be due to an asymptomatic type of infection as observed for their anti-N IgG responses (Figure 2).

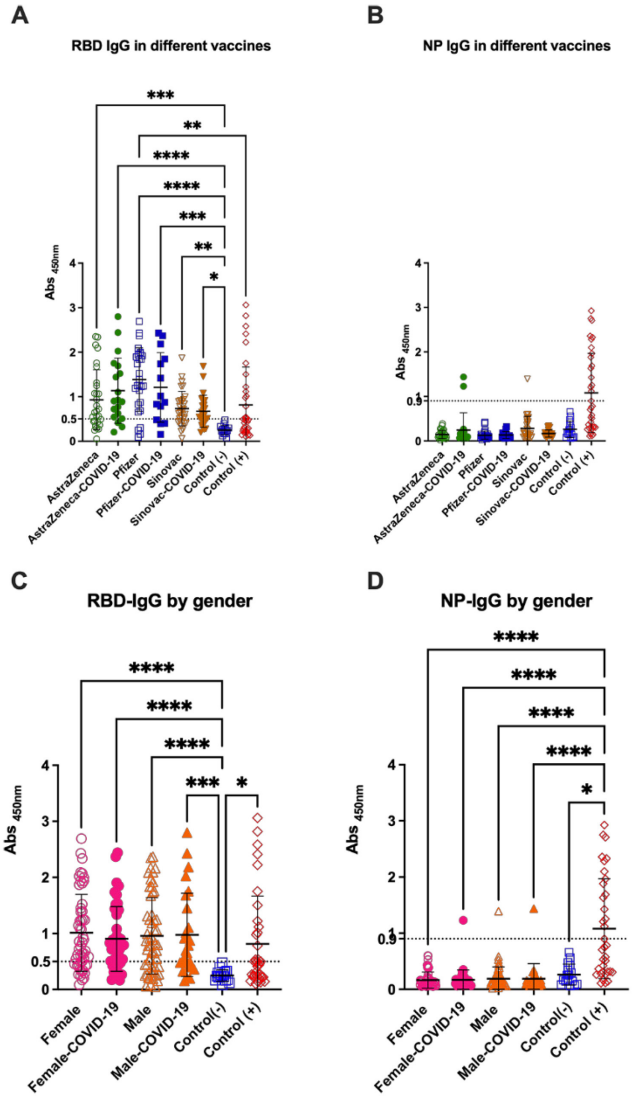
Anti-RBD, anti-N, and anti-3CLpro responses with different vaccines

After AstraZeneca, Pfizer or Sinovac vaccinations, the subjects produced mean IgG anti-RBD antibody OD values of 1.039, 1.34, and 0.68 respectively. In comparison, the mean OD value obtained by the group without COVID-19 (negative control group) was 0.24. The mean value of anti-RBD IgG produced by the AstraZeneca vaccine group was four times higher than the value of the negative control group, while the antibody titers of the Pfizer and Sinovac vaccine groups were, respectively, five and three times higher than the negative control group (Figure 3A). The three vaccinated groups (AstraZeneca, Pfizer, and Sinovac) had statistically significant differences compared to the negative control group ($p \leq 0.0001$, $p \leq 0.0001$ and $p = 0.0032$). The AstraZeneca, Pfizer, and Sinovac groups did not show significant differences in anti-RBD IgG antibody titers compared to the positive control group, except for the Pfizer non-COVID-19 subgroup where there was a difference from the positive control group ($p = 0.0027$), resulting in 1.7 times higher the value of antibodies in the Pfizer group. On the other hand,

neither vaccine group showed significant differences between individuals with or without COVID-19.

The groups of individuals with AstraZeneca, Pfizer, and Sinovac vaccinations had a mean OD of anti-NP IgG levels of 0.18, 0.12, and 0.24, respectively, being similar to the mean OD of the negative control group of 0.33 (Figure 3B). All three groups had statistically lower anti-NP IgG values than the positive control group (mean 1.07). The average value of the AstraZeneca vaccinated group was six times lower, Pfizer vaccine group was nine times lower, and Sinovac vaccine group four times lower than the value of the positive group ($p \leq 0.0001$, $p \leq 0.0001$, and $p = 0.0032$ respectively). It is important to mention that three individuals had IgG-NP values higher than the threshold (mean OD of 0.9), two of them reported having had previous SARS-CoV-2 infections, which may explain the high value for antibodies against the NP protein. However, one of the individuals did not report a previous infection, so the high value may be due to an asymptomatic infection.

Figure 3. Characterization of anti-SARS-CoV-2 IgG antibodies in vaccinated individuals.



A: Anti- receptor binding domain (RBD) IgG levels in individuals vaccinated with the AstraZeneca, Sinovac and Pfizer products who developed COVID-19 and who did not have COVID-19 prior to vaccination; the dotted line indicates the cut-off value for the antibody. B: Anti-nuclear protein (NP) IgG levels in individuals who developed COVID-19 prior to vaccination and who did not have COVID-19 and who were vaccinated with the AstraZeneca, Sinovac, and Pfizer products. C: Anti-RBD IgG levels in women and men who presented COVID-19 prior to vaccination and who did not present COVID-19. D: Anti-NP IgG levels in individuals of both genders with a diagnosis of COVID-19 prior to vaccination and without a diagnosis of COVID-19. Kruskal-Wallis analysis is presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

The analysis of IgM antibodies against the SARS-CoV-2 RBD, N, and 3CLpro proteins did not show different values between the recipients of either AstraZeneca, Pfizer, or Sinovac vaccines. However, significant differences were observed in contrast to the positive control group. Regarding the levels of anti-3CLpro IgG antibodies, there were no statistically significant differences between the AstraZeneca, Pfizer and Sinovac vaccinated groups, and the negative and positive controls (Supplementary Figure 1).

Differentiating anti-RBD, anti-N and anti-3CLpro antibody responses by gender

Regarding the anti-RBD IgG values in males and females by gender at birth, it was found that the average value in the female group with COVID-19 was 0.9 and 1.0 in the female group without COVID-19; while both male groups had an average of 1.0. No significant difference was observed between the genders (Figure 3C), which suggests that antibody production is not affected by the gender of the vaccinated individuals. The levels of both groups were statistically similar to those produced by the positive control group (0.8), which suggests that the level of IgG anti-RBD antibodies produced by vaccination in both genders is similar to the level of antibodies produced during natural infection. The levels of IgM anti-RBD did not show statistically significant differences between the different groups (Supplementary Figure 2). When analyzing the levels of IgG and IgM anti-NP in both genders, a significant difference was observed with the sera of the positive control group ($p < 0.0001$, $p < 0.05$, $p < 0.01$ respectively) (Figures 3D and Supplementary Figure 2), which aligns with fact that this type of antibody is raised in natural infection, and not by vaccinations. It is important to mention that two individuals who presented levels above the threshold (0.9) had COVID-19, while one did not report a

previous infection; so, his result may be due to an infection without symptoms. There were no significant differences in the levels of IgG anti-3CLpro between the genders and the control groups, however the IgM anti-3CLpro were statistically lower compared to the positive control group ($p < 0.01$ and $p < 0.0001$, respectively) (Supplementary Figure 2).

Discussion

Infections caused by the SARS-CoV-2 virus in the last three years have caused the largest pandemic of the century. The severity of the COVID-19 disease and the lack of specific treatment led to great efforts worldwide to contain the spread of the disease, including the development of several vaccines against SARS-CoV-2 [11,28]. However, some of the vaccines such as Ad5-nCoV approved in Mexico had not yet completed phase III trials, and uncertainty among the population was high about the efficacy and safety of the vaccines [29]. This motivated our group to carry out this work, with the objective to determine the concentrations of blood IgM and IgG responses against the RBD of the spike glycoprotein, the N protein, and the 3CLpro protein in teachers and students vaccinated with CanSinoBio, AstraZeneca, Pfizer, and Sinovac vaccines.

At the time of vaccination of teachers in Mexico with the Ad5-nCoV (CanSinoBio) vaccine, phase I and II studies had been carried out and the phase III study was under intermediate analysis; therefore, the efficacy of the vaccine was unclear and caused uncertainty in the population [29,30]. The first part of this study focused on analyzing the differences in plasma IgG antibody levels of teachers before and four weeks after immunization with the CanSinoBio vaccine considering their history of prior COVID-19 disease and age. It was observed that the increase in IgG anti-RBD antibodies was only statistically significant in individuals who suffered from COVID-19 prior to vaccination and in the age group between 40 and 49 years. In phase III clinical trials, the Ad5-nCoV vaccine showed an overall efficacy of 68.83% in all symptomatic COVID-19 infections 14 days after vaccination, and 65.28% 28 days after vaccination [30]; which suggests that the peak of efficacy is obtained between two and four weeks and then gradually decreases [31]. Likewise, it has been observed in other studies that individuals with a previous SARS-CoV-2 infection in combination with vaccination can increase the amount of neutralizing antibodies compared to those who did not have a previous infection, indicating that with a history of previous infection there is an increase in the production of antibodies [32–34] which agrees with the results obtained in this study. Teachers aged between 40-49 years had the highest levels of antibodies. This is consistent with the epidemiological data, where it was shown that people in this age range had higher levels of infection by SARS-CoV-2 [35]. In addition, Guzmán *et al*., observed that the highest production of anti-S1 IgG antibodies is associated with age groups of ≥ 41 years [36].

On the other hand, when comparing all the individuals immunized with any vaccine, we observed that the levels of anti-RBD IgG antibodies did not have significant differences between the groups with and without previous SARS-CoV-2 infection and the group of convalescent positive controls of COVID-19. This result differs from other works where it has been observed that after a second exposure to the pathogen, a phenomenon of accelerated antibody production occurs because people who had a previous natural infection followed by artificial immunization present greater antibody production [37]. However, all the vaccines studied are designed to generate antibodies against the viral spike (S) glycoprotein where the RBD is located [7,8]; the latter being, together with the N-terminal domain (NTD), the main antigens of the SARS-CoV-2 virus that exhibit neutralizing capacity [5], which is consistent with the large amount of anti-RBD IgG obtained in all groups.

In the case of IgG anti-NP and IgM anti-3CLpro antibodies, a significantly higher level was observed in the COVID-19 positive group compared to the groups vaccinated with and without pre-immunization infection. This can be explained as vaccination with BNT162b2, Ad5-nCOV, mRNA-1273, and ChAdOX1-nCoV triggers an immune response with the production of memory B cells and production of anti-spike antibodies [38]. However, a natural infection induces both a cellular and humoral response with the production of various specific antibodies against the different viral proteins, mainly surface proteins such as nsp1, nsp3, and nsp14; but also, others such as 3CLpro, E, and nucleocapsid (N) proteins [38].

When individuals were divided into groups by the type of vaccine received (AstraZeneca, Pfizer, and Sinovac) and subdivided by prior infection and not infected prior to vaccination, no significant differences in anti-RBD antibody production were observed between the groups. This may indicate that in the groups of different vaccines, antibody immunity does not change radically when the disease is previously presented naturally and when only the vaccine is received. Similarly, it can be inferred that the levels of anti-RBD IgG antibodies produced by the different

vaccines are comparable to the levels produced by natural infection [37]. In contrast, the positive control group had significantly higher anti-NP production compared to the groups vaccinated with and without prior COVID-19. This result aligns with the fact that the vaccines analyzed in this study do not induce the production of anti-NP antibodies. However, these antibodies are usually produced during natural infections by SARS-CoV-2 since it is the most abundant protein in virions and is a highly immunogenic antigen. In addition, it is also the determinant of virulence and pathogenesis [39]. On the other hand, the concentration of anti-RBD, anti-NP, and anti-3CLpro IgM antibodies was significantly higher in the positive control group compared to the different vaccine groups. This is explained by the fact that the positive group had had a recent infection at the time of sampling, so IgM levels were elevated.

The levels of anti-RBD, anti-N, and anti-3CLpro antibodies were not significantly different between males and females, which suggests that antibody production is not affected by the gender of the vaccinated individuals. This coincides with the work of Cervantes-Luévano *et al*., where they report similar antibody titers in all vaccinated men and women [33]. However, other studies report contrary results where higher levels of IgG anti-S antibodies are observed in men than in women, attributing it to the influence of hormones [36]. On the contrary, in other studies there is typically a greater immune response in women when compared to men [40]. However, some studies show that despite the fact that susceptibility to infection is almost the same between males and females, the levels of angiotensin-converting enzyme 2 (ACE2) receptor are higher in men, so SARS-CoV-2 is more successful in infecting cells through binding to its cognate receptor, possibly producing greater severity and fatality [40].

Limitations of this study include the small number of study participants and the lack of data on cellular immunity. Our findings with samples from university students and teachers from the city of Chihuahua can help to give an overview of the immunity produced after vaccination in students and teachers, helping improve vaccination strategies. This project was conducted at a public university in the state of Chihuahua, encompassing various stages of sample collection due to logistical constraints inherent in the government's vaccine distribution efforts. The Ad5-nCoV CanSinoBio vaccine was exclusively administered to teaching and administrative staff of schools during April and May 2021, whereas students received vaccination from July to March 2022. As per directives from the Mexican Public Education Secretariat, schools across Mexico were encouraged to resume in-person activities for basic education nationwide starting January 2022. Notwithstanding, the successful resumption of face-to-face activities can be attributed to the Mexican Government's vaccine campaign and the diverse range of vaccines administered to both teachers and students, which is evident in the low infection rate reported.

Conclusions

This study highlights the need for robust surveillance of humoral responses against SARS-CoV-2 in university populations, particularly in the context of vaccine administration. By establishing a novel platform for monitoring humoral responses within our university community in Chihuahua, Mexico, we have contributed valuable insights into the serological status of vaccinated teachers and students.

Our findings reveal notable disparities in IgG levels between pre-vaccination and post-vaccination serum samples, particularly among those inoculated with the CanSino vaccine. However, asymptomatic individuals did not exhibit significant changes in antibody levels post-vaccination, suggesting potential nuances in immune response dynamics. Furthermore, comparative analysis of vaccinated groups revealed no significant differences in anti-RBD IgG antibody titers, except for a Pfizer subgroup where increased antibody levels were observed. Importantly, previous COVID-19 infection did not significantly influence antibody responses among vaccine recipients.

These results elucidate the current landscape as face-to-face activities resume amidst ongoing vaccination efforts. Moving forward, continued monitoring of humoral responses will be pivotal in gauging vaccine efficacy and informing public health strategies within our university community and beyond.

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References

1. Post N, Eddy D, Huntley C, I van Schalkwyk MC, Shrotri M, LeemanID D, Rigby S, Williams S V, BerminghamID WH, Kellam P, Maher J, Shields AM, Amirthalingam G, Peacock SJ, IsmailID SA (2020) Antibody response to SARS-CoV-2 infection in humans: a systematic review. PLoS One 15: e0244126. doi: 10.1371/journal.pone.0244126.
2. World Health Organization (WHO) (2023) WHO Coronavirus (COVID-19) dashboard with vaccination data. Available: https://covid19.who.int/. Accessed: 11 May 2023.
3. Lemay DJ, Bazelais P, Doleck T (2021) Transition to online learning during the COVID-19 pandemic. Comput Hum Behav Reports 4: 100130. doi: 10.1016/j.chbr.2021.100130.
4. Klasse PJ, Nixon DF, Moore JP (2021) Immunogenicity of clinically relevant SARS-CoV-2 vaccines in nonhuman primates and humans. Sci Adv 7: 1–22. doi: 10.1126/sciadv.abe8065.
5. Rodríguez-Hernández C, Sanz-Moreno JC (2020) Immunity against SARS-CoV-2: moving towards vaccination. Off J Spanish Soc Chemother 33: 392–398. [Article in Spanish]. doi: 10.37201/req/086.2020.
6. Basta N, Moodie E, McGill UCVTT (2022) COVID-19 vaccine development and approvals tracker. Available: https://covid19.trackvaccines.org/. Accessed: 15 May 2023
7. Centers for Diseases Control and Prevention (2022) Overview of COVID-19 vaccines. CDC. Available: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/overview-COVID-19-vaccines.html. Accessed: 9 May 2023.
8. Mendonça SA, Lorincz R, Boucher P, Curiel DT (2021) Adenoviral vector vaccine platforms in the SARS-CoV-2 pandemic. npj Vaccines 6: 97. doi: 10.1038/s41541-021-00356-x.
9. Higdon MM, Wahl B, Jones CB, Rosen JG, Truelove SA, Baidya A, Nande AA, ShamaeiZadeh PA, Walter KK, Feikin DR, Patel MK, Knoll MD, Hill AL (2022) A systematic review of COVID-19 vaccine efficacy and effectiveness against SARS-CoV-2 infection and disease. Open Forum Infectious Diseases, 9: ofac138. doi: 10.1101/2021.09.17.21263549.
10. Lafon E, Jäger M, Bauer A, Reindl M, Bellmann-Weiler R, Wilflingseder D, Lass-Flörl C, Posch W (2022) Comparative analyses of IgG/IgA neutralizing effects induced by three COVID-19 vaccines against variants of concern. J Allergy Clin Immunol 149: 1242–1252. doi: 10.1016/j.jaci.2022.01.013.
11. Zhu FC, Guan XH, Li YH, Huang JY, Jiang T, Hou LH, Li JX, Yang BF, Wang L, Wang WJ, Wu SP, Wang Z, Wu XH, Xu JJ, Zhang Z, Jia SY, Wang B Sen, Hu Y, Liu JJ, Zhang J, Qian XA, Li Q, Pan HX, Jiang HD, Deng P, Gou JB, Wang XW, Wang XH, Chen W (2020) Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 396: 479–488. doi: 10.1016/S0140-6736(20)31605-6.
12. SE (Secretaria de Salud) (2020) COVID-19 vaccination begins in Mexico | Health Council| Mexican Government | gob.mx. Available: https://www.gob.mx/salud/prensa/266-arranca-vacunacion-contra-covid-19-en-mexico?idiom=es. Accessed: 15 May 2023. [Article in Spanish].
13. SE (Secretaria de Salud) (2022) Authorized COVID-19 vaccines | Federal Commission for the Protection against Sanitary Risks| Mexican Government | gob.mx. https://www.gob.mx/cofepris/acciones-y-programas/vacunas-covid-19-autorizadas. Accessed 15 May 2023. [Article in Spanish].
14. Muñoz-Valle JF, Sanchez-Zuno GA, Matuz-Flores MG, Hernández-Ramírez CO, Díaz-Pérez SA, Baños-Hernández CJ, Turrubiate-Hernández FJ, Vega-Magaña AN, Jorge H-B, Hern J (2022) Efficacy and safety of heterologous booster vaccination after Ad5-nCoV (CanSino Biologics) vaccine: a preliminary descriptive study. Vaccines 10: 1–10. doi: 10.3390/vaccines10030400.
15. SEP (Secretaria de Educación Pública) (2021) |SEP Bulletin No. 102: The vaccination process for teaching staff successfully concludes in 30 states across the country | Mexican Government | gob.mx. Available: https://www.gob.mx/sep/es/articulos/boletin-sep-no-102-concluye-exitosamente-el-proceso-de-vacunacion-a-personal-docente-en-30-entidades-del-pais?idiom=es. Accessed: 15 May 2023. [Article in Spanish].
16. SEP (Secretaria de Educación Pública) (2022) The SEP declares readiness for the return to in-person classes after the winter vacation period | Council of Public Education | Mexican Government | gob.mx. Available: https://www.gob.mx/sep/es/articulos/boletin-sep-no-1-se-declara-lista-la-sep-para-el-regreso-a-clases-presenciales-tras-el-periodo-vacacional-de-invierno?idiom=es. Accessed: 9 May 2023. [Article in Spanish].
17. SEP (Secretaria de Educación Pública) (2021) Bulletin No. 77: Vaccination of teachers, administrative staff, and support personnel in public and private schools in Tamaulipas begins tomorrow. | Council of Public Education | Mexican Government | gob.mx. Available: https://www.gob.mx/sep/articulos/boletin-no-77-manana-inicia-la-vacunacion-de-docentes-personal-administrativo-y-de-apoyo-de-escuelas-publicas-y-privadas-en-tamaulipas?idiom=es. Accessed: 15 May 2023. [Article in Spanish].
18. UACH (Universidad Autónoma de Chihuahua) (2021) UACH Students will return only for laboratories, workshops, and practical sessions. https://uach.mx/noticias/2021/8/4/regresaran-estudiantes-de-la-uach-solo-a-laboratorios-talleres-y-practicas/. Accessed 8 May 2023, [Article in Spanish].
19. UACH (Universidad Autónoma de Chihuahua) (2022) UACH announces hybrid return to classes on January 24, 2022. Available: https://uach.mx/noticias/2022/1/4/anuncia-uach-regreso-a-clases-hibrido-este-24-de-enero-2022/. Accessed: 8 May 2023. [Article in Spanish].
20. Sabater-Vidal S, Tirado-Balaguer MD, Soria-Martín R, Arnedo-Pena A, Gil-Galdón A, Bellido-Cambrón MC, Moreno-Muñoz MR (2022) Post-vaccination antibodies against SARS-CoV-2 in a cohort of general hospital workers. 25: 358-378. [Article in Spanish]. doi: 10.12961/aprl.2022.25.04.03.
21. Muro A, Belhassen-García M, Bellido JLM, Juanes HL, Vicente B, Pendones J, Adserias J, Hernández GS, Rosa MR, Villardón JLV, Burguillo J, Andaluz JL, Oterino JAM, Criado FJG, Barbero F, Morales AI, Villardón PG, Sarmiento RG (2021) Seroprevalence of SARS-CoV-2 antibodies and factors associated with seropositivity at the University of Salamanca: the DIANCUSAL study. J Clin Med 10: 3214. doi: 10.3390/jcm10153214.
22. Vusirikala A, Whitaker H, Jones S, Tessier E, Borrow R, Linley E, Hoschler K, Baawuah F, Ahmad S, Andrews N, Ramsay M, Ladhani SN, Brown KE, Amirthalingam G (2021)

Seroprevalence of SARS-CoV-2 antibodies in university students: cross-sectional study, December 2020, England. J Infect 83: 104. doi: 10.1016/j.jinf.2021.04.028.
23. Ladhani SN, Ireland G, Baawuah F, Beckmann J, Okike IO, Ahmad S, Garstang J, Brent AJ, Brent B, Walker J, Aiano F, Amin-Chowdhury Z, Letley L, Flood J, Jones SEI, Kall M, Borrow R, Linley E, Zambon M, Poh J, Lackenby A, Ellis J, Amirthalingam G, Brown KE, Ramsay ME (2021) SARS-CoV-2 infection, antibody positivity and seroconversion rates in staff and students following full reopening of secondary schools in England: a prospective cohort study, September-December 2020. eClinicalMedicine 37: 100948. doi: 10.1016/j.eclinm.2021.100948.
24. Muñoz-Medina JE, Grajales-Muñiz C, Salas-Lais AG, Fernandes-Matano L, López-Macías C, Monroy-Muñoz IE, Coy-Arechavaleta AS, Palomec-Nava ID, Duque-Molina C, Madera-Sandoval RL, Rivero-Arredondo V, González-Ibarra J, Alvarado-Yaah JE, Rojas-Mendoza T, Santacruz-Tinoco CE, González-Bonilla CR, Borja-Aburto VH (2021) SARS-CoV-2 IgG antibodies seroprevalence and sera neutralizing activity in Mexico: a national cross-sectional study during 2020. Microorganisms 9: 1–15. doi: 10.3390/microorganisms9040850.
25. Basto-Abreu A, Carnalla M, Torres-Ibarra L, Romero-Martínez M, Martínez-Barnetche J, López-Martínez I, Aparicio-Antonio R, Shamah-Levy T, Alpuche-Aranda C, Rivera JA, Barrientos-Gutierrez T, Cuevas-Nasu L, Gaona-Pineda EB, Ávila-Arcos MA, Reyes-Sánchez F, Torres-Álvarez R, López-Olmedo N, Vidaña-Perez D, González-Morales R, Barrera-Nuñez D, Perez-Ferrer C, Gaspar-Castillo C, Stern D, Canto-Osorio F, Sanchez-Pájaro A (2022) Nationally representative SARS-CoV-2 antibody prevalence estimates after the first epidemic wave in Mexico. Nat Commun 13: 589. doi: 10.1038/s41467-022-28232-9.
26. Richardson V, Camacho M, Bautista A, Martínez L, Castro L, Cruz V, Gharpure R, Lafond K, Yau T, Azziz-Baumgartner E, Hernández M (2022) Vaccine effectiveness of CanSino (Adv5-nCoV) COVID-19 vaccine among childcare workers - Mexico, March-December 2021. Clin Infect Dis 75 Suppl 2: S167–S73. doi: 10.1101/2022.04.14.22273413.
27. Alcorta-Nuñez F, Pérez-Ibave DC, Burciaga-Flores CH, Garza MÁ, González-Escamilla M, Rodríguez-Niño P, González-Guerrero JF, Alcorta-Garza A, Vidal-Gutiérrez O, Ramírez-Correa GA, Garza-Rodríguez ML (2023) SARS-CoV-2 neutralizing antibodies in Mexican population: a five vaccine comparison. Diagnostics 13: 1194. doi: 10.3390/diagnostics13061194.
28. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL (2020) Safety and efficacy of the BNT162b2 mRNA COVID-19 Vaccine. New Engl J Med 383: 2603–2615. doi: 10.1056/NEJMoa2034577.
29. Hernández-Bello J, Morales-Núñez JJ, Machado-Sulbarán AC, Díaz-Pérez SA, Torres-Hernández PC, Balcázar-Félix P, Gutiérrez-Brito JA, Lomelí-Nieto JA, Muñoz-Valle JF (2021) Neutralizing antibodies against SARS-CoV-2, anti-Ad5 antibodies, and reactogenicity in response to Ad5-nCoV (CanSino Biologics) vaccine in individuals with and without prior SARS-CoV-2. Vaccines 9: 1047. doi: 10.3390/vaccines9091047.
30. Díaz-Ortega JL (2021) Technical guide for the administration of the recombinant vaccine against the novel coronavirus (adenovirus type 5 vector) by CanSino Biologics, against the SARS-CoV-2 Virus. Available: http://clinicadeviajero.unam.mx/?p=7744. Accessed: 9 May 2023. [Article in Spanish].
31. Halperin SA, Ye L, MacKinnon-Cameron D, Smith B, Cahn PE, Ruiz-Palacios GM, Ikram A, Lanas F, Guerrero ML, Muñoz Navarro SR, Sued O, Lioznov D, Vitalina D, Parveen G, Zhu F, Leppan L, Langley JM, Barreto L, Gou J, Zhu T (2022) Final efficacy analysis, interim safety analysis, and immunogenicity of a single dose of recombinant novel coronavirus vaccine (adenovirus type 5 vector) in adults 18 years and older: an international, multicentre, randomised, double-blinded, placebo-controlled phase-3 trial. Lancet 399: 237–248. doi: 10.1016/S0140-6736(21)02753-7.
32. Cabanillas-Bernal O, Cervantes-Luevano K, Isai Flores-Acosta G, Bernáldez-Sarabia J, Licea-Navarro AF (2022) COVID-19 neutralizing antibodies in breast milk of mothers vaccinated with three different vaccines in Mexico. Vaccines (Basel) 10: 629. doi: 10.3390/vaccines10040629.
33. Cervantes-Luevano K, Espino-Vazquez AN, Flores-Acosta G, Bernaldez-Sarabia J, Cabanillas-Bernal O, Gasperin-Bulbarela J, Gonzalez-Sanchez R, Comas-Garcia A, Licea-Navarro AF (2022) Neutralizing antibodies levels are increased in individuals with heterologous vaccination and hybrid immunity with Ad5-nCoV in the north of Mexico. 1273: 1–16. doi: 10.1371/journal.pone.0269032.
34. Wise J (2021) COVID-19: people who have had infection might only need one dose of mRNA vaccine. BMJ 372: n308. doi: 10.1136/bmj.n30.
35. Pan American Health Organization/World Health Organization (PAHO/WHO) (2021) Health outcomes disaggregated by sex in relation to the COVID-19 pandemic in the region of the Americas. Available: https://www.paho.org/es/documentos/resultados-salud-desglosados-por-sexo-relacion-con-pandemia-covid-19-region-americas. Accessed: 9 May 2023. [Article in Spanish].
36. Guzmán-Martínez O, Guardado K, Varela-Cardoso M, Trujillo-Rivera A, Marín-Hernández A, Cristina Ortiz-León M, Gómez-Ñañez I, Gutiérrez M, Espinosa R, Luz Sampieri C, Ramos C, Vanesa López-Guerrero D, Zenteno-Cuevas R, Isael Pérez-Carreón J, Canales-Vázquez G, Montero H, Montero hmontero H, Mendoza C, de Alta Especialidad C, Lucio R (2022) Generation and persistence of S1 IgG and neutralizing antibodies in post-COVID-19 patients. Infection, 50: 447–456. doi: 10.1007/s15010-021-01705-7.
37. Prendecki M, Clarke C, Brown J, Cox A, Gleeson S, Guckian M (2021) Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine. Lancet 397: 1178–1181. doi: 10.1016/S0140-6736(21)00502-X.
38. Enjuanes L, Zuñiga S (2016) Molecular basis of coronavirus virulence and vaccine development. Adv Virus Res 96: 245–286. doi: 10.1016/bs.aivir.2016.08.003.
39. Wu W, Cheng Y, Zhou H, Sun C, Zhang S (2023) The SARS-CoV-2 nucleocapsid protein: its role in the viral life cycle, structure and functions, and use as a potential target in the development of vaccines and diagnostics. Virol J 20: 6. doi: 10.1186/s12985-023-01968-6.
40. Flanagan KL, Fink AL, Plebanski M, Klein SL (2017) Sex and gender differences in the outcomes of vaccination over the life course. Annu Rev Cell Dev Biol 33: 577–599. doi: 10.1146/annurev-cellbio-100616-060718.
41. Mukherjee S, Pahan K (2021) Is COVID-19 gender-sensitive? J Neuroimmune Pharmacol 16: 38–47. doi: 10.1007/s11481-020-09974-z.

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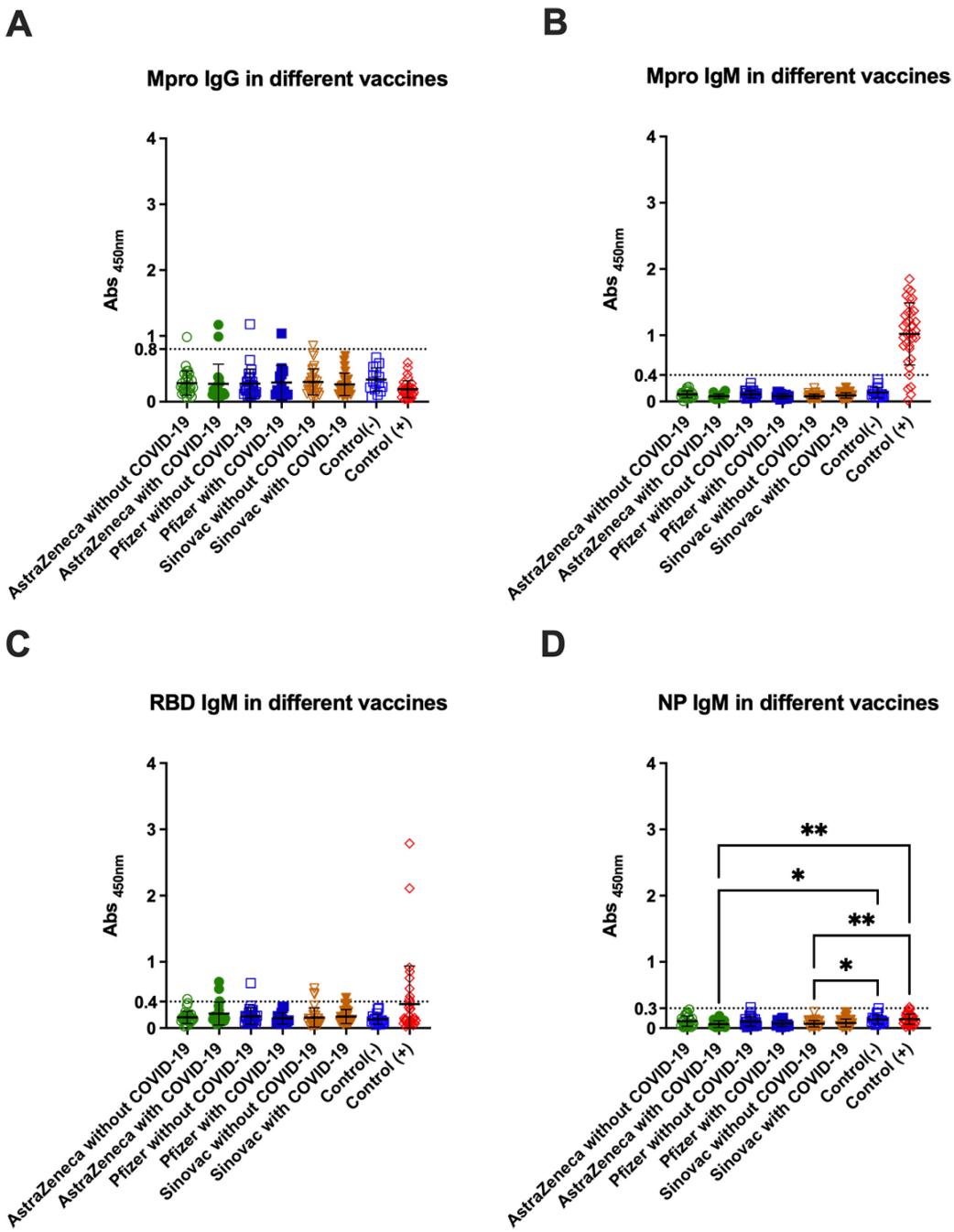
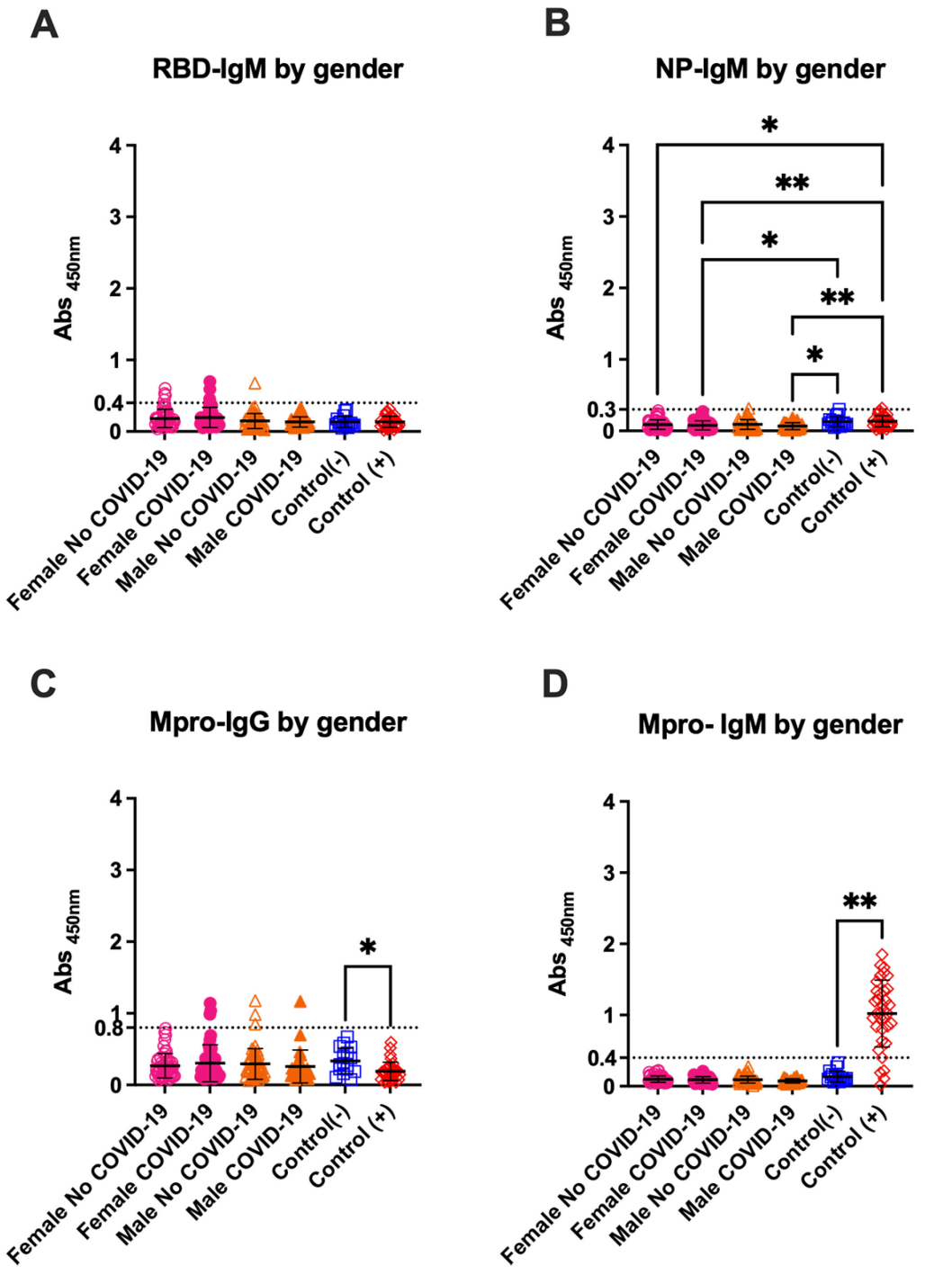
Annex – Supplementary Items

Supplementary Figure 1. Characterization of anti-SARS-CoV-2 antibodies in vaccinated individuals.



A: Anti-3C-like proteinase (3CLpro) IgG levels in individuals vaccinated with AstraZeneca, Sinovac and Pfizer products who developed COVID-19 and who did not have COVID-19 prior to vaccination. The dotted line indicates the cut-off value for the antibody; B: Anti-3CLpro protein IgM levels in individuals who developed COVID-19 prior to vaccination and who did not have COVID-19 and who were vaccinated with AstraZeneca, Sinovac, and Pfizer products; C: Anti-receptor binding domain (RBD) IgM levels in groups with AstraZeneca, Sinovac and Pfizer vaccinations with COVID-19 prior to vaccination and who did not report COVID-19; D: Anti-nuclear protein (NP) IgM levels in groups with AstraZeneca, Sinovac and Pfizer vaccinations with a diagnosis of COVID-19 prior to vaccination and without a diagnosis of COVID-19. Kruskal-Wallis analysis is presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplementary Figure 2. Characterization of anti-SARS-CoV-2 IgM and IgG antibodies in different genders.



A: Anti- receptor binding domain (RBD) IgM levels among females and males who developed COVID-19 and who did not have COVID-19 prior to vaccination; the dotted line indicates the cut-off value for the antibody; B: Anti-nuclear protein (NP) IgM levels by gender who developed COVID-19 prior to vaccination and who did not have COVID-19; C: Anti-3C-like proteinase (3CLpro) IgG levels in women and men who presented COVID-19 prior to vaccination and who did not present COVID-19; D: Anti-Mpro IgM levels in individuals of both genders with a diagnosis of COVID-19 prior to vaccination and without a diagnosis of COVID-19. Kruskal-Wallis analysis is presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.