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Research Article

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Acquired Immunity and Effects of SARS-CoV-2 Vaccination in Healthcare Work-ers and Nursing Students. Post-Booster Follow-Up

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Abstract

Introduction: Discussions on COVID-19 vaccine efficacy and the duration of immunity are relevant to health policy intervention. Aims: To assess adherence to SARS-CoV-2 vaccination in healthcare workers (HCWs) and nursing students (NS). To determine IgG levels after booster doses and to identify effects of vaccination. Methods: Observational follow-up study on the impact of COVID-19 and vaccine efficacy in 2021 and 2022. The study population comprised HCWs and NS from Albacete (Spain). The sample size was 179 HCWs and 120 NS. Data was gathered using a self-administered questionnaire and venous blood samples 6 months after full vaccination and 6 months after the booster dose, in which IgG levels against SARS-CoV2 were measured. Univariate statistical analyses and bivariate analyses were performed using SPSS (28.0). The project was ap-proved by the Ethics and Clinical Research Committee of Albacete. Results: A total of 229 individuals completed the questionnaires and participated in the initial analyses (148 HCWs and 81 NSs). Adherence to the SARS-CoV-2 vaccination regimen was high (100% in NS and 97% in HCWs). Booster doses were received by 94% of the sample. Homologous vaccines were received by 29% and het-erologous vaccines by 71%. IgG levels: 6 months after full vaccination, seropositivity was 100% and mean IgG levels were 3,017.2 AU/ml in HCWs and 2,484.62 AU/ml in NS ($p<0.001$). At 6 months after the booster dose the mean IgG levels were 25,789.34 AU/ml in PS and 17,155.07 AU/ml in NS ($p<0.001$). Adverse effects of the vaccines were mild and local, with some present in a high proportion of those vaccinated. Conclusions: After vaccination, antibody levels were positive at 6 months and increase considerably after the booster dose, remaining high 6 months later. No serious side effects were reported, but a high incidence of mild effects, both general and local, was observed.

Keywords: Healthcare Workers, SARS-CoV-2 Vaccine, Nursing Students, COVID-19 Vaccines, Vaccination, Booster Dose, Heterologous and Homologous COVID-19 Vaccine Regimens, Side Effects

Introduction

In May 2023, the World Health Organisation (WHO) declared the end of the "public health emergency of international concern" after more than three years of the COVID-19 pandemic, caused by the SARS-CoV-2 virus [1]. Mass vaccination is the strategy attributed with bringing the pandemic under control and its most devastating effects: high mortality rates and the frequency of severe cases.

The impact of the virus has slowed down globally in the last six months. The WHO reported 768,187,096 confirmed cases of COVID-19 at the end of June 2023, including 6,945,714 deaths. At that time, the WHO was still reporting more than 200,000 new cases per week. Three months later, on 30 November 2023, the WHO reported 772,052,752 confirmed cases of COVID-19, including 6,985,278 deaths, with new cases falling to 1,163 in the last week of November. Meanwhile, in Europe, a total of 276,545,745 cases had been confirmed by June 2023, rising to 277,810,273 cases by the end of November. According to WHO data, a total of 13,595,583,125 doses of vaccine had been administered by the same date [2].

Healthcare workers suffered greatly during the emergency and their commitment was key in dealing with the pandemic, administering vaccines, caring for the sick and supporting research in various fields [3]. Nursing students are a group

of young adults that occupy a special position among health personnel, and several cohorts of graduates felt the effects of the pandemic in their training. The main effects were the suppression of practical activities for several months, a change to online teaching during the confinement and blended learning in the following year [4].

Spain was one of the first countries in Europe to be affected by the COVID-19 pandemic and was obliged to adopt measures to both prevent infection and to increase care capacity at hospital level, in particular to enhance the capacity of critical care units. These changes involved health workers at both primary care and hospital level. Many of the measures implemented to respond to the pandemic remain in place and will be consolidated with EU funding, among which are the creation of a new public health authority and improved epidemiological surveillance [5].

The swift availability of vaccines and the widespread vaccination coverage achieved in many countries around the world made it possible to overcome, within two years, the most severe impacts of SARS-CoV-2 in terms of morbidity and mortality. In addition, vaccination has prevented the collapse of health systems. Although agreement exists on the effectiveness of vaccines in preventing COVID-19 mortality and reducing severe cases requiring hospitalisation, greater doubts surround the effect of vaccines in preventing transmission [6].

Myriad studies have assessed vaccine response in healthcare workers from the administration of the first doses in December 2020 to the present. Humoral response (evaluated by measuring IgG and neutralising antibodies) and vaccine efficacy (comparing infection rates between vaccinated and unvaccinated individuals or between pre- and post-vaccination periods) are the two indicators typically considered for vaccine evaluation in observational studies [7]. Studies have documented the greater robustness and duration of hybrid immunity, i.e., that generated in vaccinated individuals who have previously acquired natural immunity [8]. Discussion continues, however, on the duration of this hybrid immunity and its efficacy against variants such as Delta: B.1.617 or VUI-21APR-01 and Omicron B.1.1.529 [9,10].

The dynamic evolution of the COVID-19 pandemic situation, with the emergence of variants of concern, such as the abovementioned Delta and Omicron, has driven a continuous review and evaluation of medications by national and international regulators [11]. The human response to SARS-CoV-2 vaccines is still far from being fully characterised, and thus further studies following the evolution of the humoral response are recommended [12]. Debates continue, however, on the efficacy of booster doses, the advisability of implementing them on an annual seasonal basis, like the flu vaccine, the best combination of vaccines and who to consider the most at-risk or vulnerable groups that should periodically be given these booster doses [13].

Since the end of 2020, two types of COVID-19 vaccines have been available: messenger RNA vaccines (Moderna and Pfizer) and adenoviral vaccines (AstraZeneca and Jansen). These were the first to be authorised by the European Medicines Agency, but others have since appeared with a slightly different composition (inactivated virus or protein). The first Spanish vaccine is called Hipra (Bimervax) and was authorised in March 2023. Based on protein, it is indicated as a booster dose for persons aged over 16 years who received an mRNA vaccine at primary vaccination [14].

Discussions on vaccine efficacy and duration of immunity are central to health policy interventions, including the timing of vaccine boosters and the most advisable schedules, and thus some of the most recent studies refer to follow-up after the third or fourth booster dose (7) [15]. Studies have also focused on the immunogenicity of heterologous vaccination, which combines different types of vaccines, versus homologous vaccination, in which the same vaccine is used for all doses, including the booster [16].

This study addresses the duration of humoral immunity from six months after the full vaccination schedule to six months after the booster dose in a HCWs with and without hybrid immunity. We explore vaccination adherence in healthcare workers, the effects of vaccines and factors affecting levels immunity, both those related to individuals and those associated with the types of vaccines received and how they are combined in the different doses.

2. AIMS

2.1 General

To determine the level of acquired immunity generated by SARS-Cov-2 vaccination in healthcare workers.

2.2 Specific

- To monitor vaccine response after booster doses.
- To identify variations in the immune response according to the combination of vaccines administered (homologous and heterologous).
- To identify differences and establish factors related to immunity in both population groups.
- To identify the side effects of the vaccines against SARS-CoV-2 and their evolution according to dose and vaccination schedule.

3. Methods

Design: This was an observational follow-up study on the efficacy of vaccines in healthcare workers during 2021 and 2022. It addresses the question of COVID-19 incidence, acquired immunity, vaccination adherence and the impact of SARS-CoV-2 vaccines in healthcare workers and nursing students. The study was carried out in two towns in the region of Castilla-La Mancha (Spain).

Population: Workers from the health area of Almansa (HCWs) and nursing students (NS), with an initial population of 620 individuals from the Integrated Care Management (ICM) of Almansa and 230 students from the Faculty of Nursing in Albacete (2nd-, 3rd- and 4th-year students). The sample size was calculated for a confidence level of 95% NC, an estimated error of ±0.03 and an estimated proportion of 95%. To this number, we added 10%, estimating possible losses. The resulting sample size was 179 persons for HCWs and 120 for NS. Simple random sampling (SRS) was carried out, using the

corporate mailing lists provided by the ICM of Almansa, and the persons selected were invited to participate in the study, by means of an informed consent form. Those that failed to respond or declined to participate were replaced by others from the same population. The final sample of persons recruited and who took part participating in the study comprised 150 individuals from the ICM of Almansa (representing 83.8% of the initial sample) and 81 nursing students (67% of the initial sample, selected in this case in a convenience sample). In the student population, participation was requested of those who had had contact with patients, and participation was thus limited to students enrolled in the 2nd, 3rd and 4th years in 2021, since 1st-year students receive no practical training at clinical centres.

Sources of information: Data were gathered using a self-report questionnaire and a venous blood sample to determine IgG levels collected 6 months after full vaccination and 6 months after the booster dose. The fieldwork lasted 18 months, from June 2021 to October 2022.

Study variables: sociodemographic, clinical, epidemiological and employment data, SARS-CoV-2 vaccination, vaccine adherence, side effects and IgG levels against SARS-CoV2 at 6 months and 6 months after the third dose.

The questionnaire was designed by the research team, using WHO-validated classification criteria. It was subjected to the review of experts and an initial pilot test was performed to ensure the items were easily understandable. The English version of the questionnaire may be requested from the authors. The questionnaire was administered at the start of the study, with certain items being updated at the follow-up measurement or being recorded according to the time of the study (e.g. date and type of vaccination, SARS-CoV-2 infection or change of habits).

Determination of SARS-CoV2 serum IgG levels: the Alinity i SARS-CoV-2 IgG II Quant assay (Abbott ®) was performed on all samples. It is based on a chemiluminescent microparticle immunoassay (CMIA) that qualitatively and quantitatively determines IgG antibodies against the receptor binding domain (RBD), located in spike protein subunit 1 (S1) of SARS-CoV-2. The sensitivity reported by the laboratory is 100% and specificity is 99.9%. The unit of measurement is AU/ml (arbitrary units per millilitre). A positive antibody response was considered at an IgG level ≥ 50 AU/ml [17].

Analysis: A descriptive univariate analysis was performed using measures of central tendency and dispersion for the continuous variables and absolute and relative frequencies for the categorical variables. We calculated 95% confidence intervals. The main variables were tested for normality distribution. The bivariate analysis was conducted using the corresponding tests: Chi-squared for comparison of proportions, t-Student for comparison of means in two groups, ANOVA for comparison of means in n-groups, the non-parametric Kruskal-Wallis and Mann-Whitney tests when the conditions for the use of parametric tests are not met and the Wilcoxon signed-rank test for paired samples. Due to the non-normal distribution of IgG values, logarithms were taken, and geometric means were calculated, and then bivariate analysis and group comparisons with parametric tests were performed (Student's t-test, ANOVA…) or the bivariate analysis was conducted using the non-parametric Kruskal-Wallis and Mann-Whitney tests. Bilateral comparisons were used with a significance level of $p<0.05$. The data were processed and analysed using SPSS® IBM 28.0.

Ethical considerations: The project was approved by the Ethics and Clinical Research Committee of the corresponding health area. It was also approved by the SESCAM (Castilla-La Mancha Public Health Service), Code 2021-27 prospective observational study, on 11 June 2021. It was published in the Spanish Registry of Clinical Studies (ReeC) as is compulsory for studies with this type of design. All the participants were informed that personal data would remain confidential, and gave their signed consent for participation. The samples were anonymised. The researchers declare they have no conflicts of interest.

4. Results

A total of 229 individuals completed the questionnaires and participated in the initial analyses (148 HCWs and 81 NS). Table 1 shows the descriptive data for the population, exposure to COVID-19 and the vaccines administered. The basic demographic characteristics of the population are as follows: the mean age of HCWs is 46.45 years; 76% female; 85.1% work at hospitals; 34% nurses, 28% practical nurses and 13.6% doctors with an average professional experience of 17.9 years. The mean age of the NS is 21.85 years; 90.1% female; the distribution by year of enrolment is 54.3% from the 3rd year, 19.8% from the 2nd year and 25.9% from the 4th year. The population at the last follow-up is 187 (81.7% of the initial population). Although the students shared exposure to occupational risk with HCWs, the time and intensity of exposure were different. Therefore, the students represent a subgroup within the HCWs with different systemic characteristics for the study of SARS-CoV-2, its incidence and prevention. It was thus decided to present the two groups results separately.

	Total population (n=229)	HCWs (148)	NS (81)
Age (years):			
Min- Max	18.88 – 65.92	24.11 – 65.92	18.88 - 46.62
Mean (SD)	37.97 (14.62)	46.45 (9.89)	21.85 (4.64)
Sex (% Female)	81.2	76	90
Occupational exposure to COVID-19 patients (%)	62.4	83.1	42.5
CoVID-19 infection (%) Had COVID-19 in 2020	22.6	25.5	17.3
Infection 2021-22 (18m)	41.9	40.9	40.3
Vaccinated against SARS-CoV-2 Schedule (%) Received 3rd dose (booster) (%)	98.7 94.3	97.3 93.6	100.0 95.4
Vaccine adherence (%)			
Previous influenza vaccination (*) Influenza vaccination 2020-21	43.0 59.7	51.8 62.4	27.5 55.0
Types of vaccines received (%):			
Pfizer/BioNTech	82.5	100.0	51.9
AstraZeneca	16.6	0.0	45.7
Moderna	0.9	0.0	2.5
Types of vaccines received as **booster doses (%**			
Pfizer/BioNTech	46.3	17.6	93.5
AstraZeneca	1.2	1.0	1.6
Moderna	52.4	81.4	4.8
Note: HCWs = Healthcare workers; NS= Nursing students. (*) Significant differences. Pearson's chi-squared=12.63; p <0.001			

Table 1: Description of population. Exposure to COVID-19 and vaccination in HCWs and NS

4.1 Occupational Exposure to COVID-19 and Vaccination

Of the HCWs, 83.1% had contact with actively infected individuals and the work positions of 21.9% were changed in 2020. In the NS, exposure to actively infected individuals during clinical practice was lower (42.5%).

In the initial vaccination schedule, 96.7% of the HCWs were vaccinated (100% with Pfizer/BioNTech) and 100% of the NS were vaccinated (Pfizer 51.9%, AstraZeneca 45.7% and Moderna 2.5%). As regards booster doses, Moderna and Pfizer/BioNTech vaccines were primarily administered. Table 1 shows the descriptive data for the population, exposure to COVID-19 and the vaccines administered.

High adherence to the SARS-CoV-2 vaccination schedule can be observed (100% in NS and 97% in HCWs in the initial two-dose schedule), although 2% of HWCs delayed the first dose. In the case of the booster, the number of individuals vaccinated with this dose fell slightly below 100% in both groups (93-95%).

When compared with adherence to the influenza vaccine, which has long been recommended for healthcare personnel, adherence is lower, although with differences between the groups, being higher in the HCW group and somewhat lower in the NS group. There is an increase in the percentage of NS vaccinated against influenza in the 2020-21 campaign compared to previous years.

4.2 Levels of Acquired Immunity and Changes After the Booster Dose

IgG levels and vaccine effects: At 6 months after full vaccination, 100% seropositivity and mean IgG levels are 3,017.2 AU/ml in HCWs and 2,484.62 AU/ml in NS (non-significant differences). IgG levels for the overall population are 2,825.72 AU/ml on average with a median of 1,182 AU/ml and values ranging from 40 AU/ml (minimum) to 36.644 (maximum).

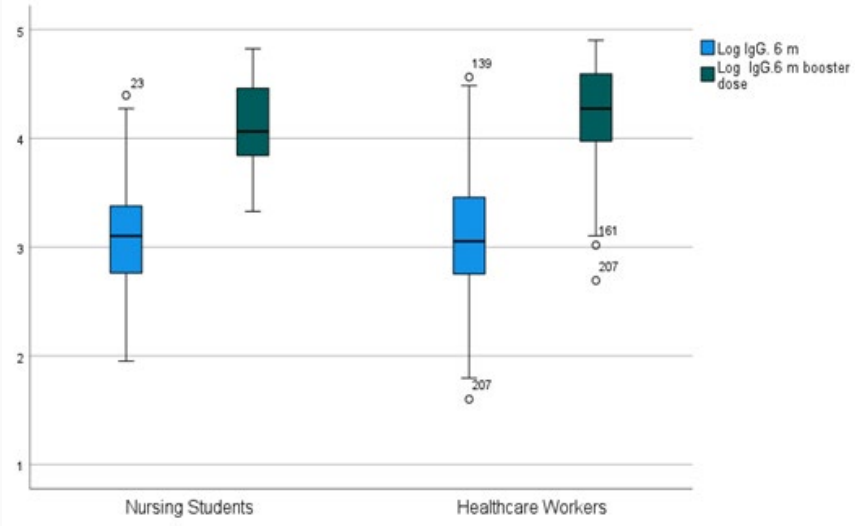
At data collection to assess IgG levels 6 months after the booster dose, the available sample comprised 187 participants, representing an 18.3% loss with respect to the initial population. At 6 months post-booster, mean IgG levels show much higher values, with mean IgG values of 22,741 AU/ml (6.9 times the mean of the previous measurement); with a median of 16,277.4 AU/ml and values ranging from 494.4 to 80,000 Au/ml.

	Healthcare workers (HW) Ig G 6 m vacc	Nursing students (NS) Ig G 6 m vacc	Healthcare workers (HW) Ig G 6 m after booster	Nursing students (NS) Ig G 6 m after booster
Total participants (n)	141	79	121	66
Mean IgG (95% CI)	3,017,40 (2124.48 -3,910.31)	2,484.62 (1,590.13 -3,379.10)	25,789.34 (23,832.97 – 27,445.71)	17,155.07 (15,474.85 – 18,835.29)
SD	5,362.91	3,993.45	21,520.13	13,650.19
Minimum value	62.6	87.7	494.4	2,132.8
Maximum value	36,644.7	24,91.,4	80,000.0	66,829.9
Log10 IgG Mean (SD)	3.148 (0.5)	3.087 (0.5)	4.238 (0.4)	4.091 (0.3)
The mean Log IgG at 6 months after full vaccination is compared: Mean Log IgG at 6 months between the two groups: t- Student= -0.862 p =0.195			**The mean Log IgG at 6 months after the booster dose is compared:** t- Student= -2.276 p =0.02 Significant differences	

Table 2. Acquired immunity: IgG levels in vaccinated population 6 months after full vaccination and 6 months after the booster dose.

An analysis of the variations in the two study populations shows the mean 6-months post- booster IgG levels are 25,789.34 AU/ml in HCWs and 17,155.07 AU/ml in NS (significant differences p<0.001). These variations, which can be seen in Table 2, represent 6.9 times the mean IgG in nursing students and 8.5 times the mean IgG data in healthcare workers, compared to the measurements taken 6 months after full vaccination. The mean IgG values with the booster dose shows a Sharp increase. The students, nonetheless, present significantly lower mean values than the healthcare professionals.

The first booster dose raised IgG levels by 590% to 755% in the two study groups. Graph 1 shows these changes and provides data on both the concentration of the average values (log IgG) and their dispersion in both groups, differences that are statistically significant, as shown in Table 2.



Graph 1: *Differences between IgG levels (Log IgG) against SARS-CoV-2 in NS and HCWs, measured 6 months after full vaccination and 6 months after the booster dose.*

We also compared the mean IgG values against SARS-CoV-2 in the repeated measures of individuals in both groups at 6 months after full vaccination and the measures at 6 months after the booster dose. This comparison, performed using the Wilcoxon signed-rank test for paired samples, also reflects the notable differences in the results: Z= -8.75, p<0.001 for HCWs and Z=-6.574, p<0.001 for NS.

5. Immunity-Related Factors

Of the factors analysed and reported in other studies, only a history of COVID-19 (so-called hybrid immunity) and smoking generated significant variations in IgG levels in the measurements taken. As shown in Table 3, neither moderate alcohol consumption nor age is associated with significant variations in measurements in either group. However, when assessing variations after the booster dose, the inverse relationship between IgG levels and smoking is not confirmed and the only association that continues to be found is a statistically significant relationship between a history of COVID-19 and a higher IgG level in the group of healthcare workers.

Sex (mean)	Healthcare workers (HCWs) (n) IgG 6 m vacc	Nursing Students (NS) (n) IgG 6 m vacc	Healthcare workers (HCWs) (n) IgG 6m booster	Nursing Students (NS) (n) IgG 6 m booster
Female Male Statistic and p-value	(106) 2,782.91 (35) 3,727.57 t-Student *=1576.50 p=0.184	(71) 2,546.62 (8) 1,934.38 Mann-Whitney U test =272.0 p=0.845	(92) 24,827.92 (29) 28,839.35 Mann-Whitney U test =1,227; p=0.516	(60) 17,942.37 (6) 9,282,37 Mann-Whitney U test=92,0; p=0.049
Smoking	**HCWs n (%); IgG 6m vacc**	**NS n (%); IgG 6m vacc**	**HCWs 6m post-booster**	**NS 6m post-booster**
Non-smokers Smokers Ex-smokers Statistic and p-value	75(50%); 3,912.23 25(16.7%); 1,267.7 42(28.0%); 2,701.7 K-Wallis= 9.916; p= 0.007	69(85%); 2,632.54 7(8.6%); 1,113.42 5(6.2%); 2,422.10 K-Wallis= 2.256; p=0.324	(56) 27,673.90 (21) 20,897.21 (22) 27,570.88 K-Wallis=1.160 p=0.560	(53) 16,133.16 (8) 23,074.57 (4) 19,495.52 K-Wallis=0.998 p=0.607
Alcohol consumption	**HCWs n (%); IgG 6m vacc**	**NS n (%); IgG 6m vacc**	**HCWs 6m post-booster**	**NS 6m post-booster**
Never Infrequent Occasional Weekly Statistic and p-value	12 (8.8%); 2,192.6 61 (48.5%); 3,437.27 40 (29.4%); 2,,680.06 16 (13.2%; 4010.66 K-Wallis= 0.891; p= 0.828	7 (8.6%); 3,679.47 20(24.7%);3,267.58 46 (59.3%) 2,118.39 6 (7.4%) 1,288.55 K-Wallis= 3.706; p= 0.295	11(11.5%);41,312.4 45 (47.9%);19,880.5 25 (27.1%);32,369.9 13 (13.5%);35,102.9 K-Wallis= 10.918; p= 0.012	8 (12,1%); 17,495.6 19 (30,3%)18,139.9 37(56,1%);19,966.9 1 (1,5%); 5,232,2 K-Wallis= 2.424; p= 0.489
Occupational exposure to COVID-19	**HCWs n, IgG 6m vacc**	**NS n; IgG 6m vacc**	**HCWs 6m post-booster**	**NS 6m post-booster**
Yes No Statistic and p-value	(107) 2,112.61 (22) 7,232.53 Mann-Whitney U test = 937.00; p=0.133	(30) 2,086.91 (41) 2,467.81 Mann-Whitney U = 599.000; p= .852	(91) 25,611.33 (20) 30,113.11 Mann-Whitney U test = 795.50; p=0.380	(24) 17,618.34 (34) 16,628.82 Mann-Whitney U test = 395.5; p=0.844
COVID-19 history	**HCWs n; IgG 6m vacc**	**NS n; IgG 6m vacc**	**HCWs 6m post-booster**	**NS 6m post-booster**
Yes No Statistic and p-value	(33) 7,520.05 (101)1,567.62 Mann-Whitney U test = 676.0; p = 0.000	(9) 3,802.72 (65) 2,326.04 Mann-Whitney U test = 139; p=0.011	(45) 31,660.37 (64) 22,735.11 Mann-Whitney U test = 1,023.5; p < 0.01	(26) 18,552,55 (39) 15,690,65 Mann-Whitney U test = 388.0; p=0.111
IgG mean values expressed in AU/ml – The comparison statistic and p-value are shown. (*) the comparison was performed using the mean log of IgG. Source: own preparation. Population from the ICM of Almansa (HCWs) and the Albacete UCLM Faculty of Nursing (NS).				

Table 3: Shows these differences as well as the results for the level of antibodies against SARS-CoV-2 in our participants, according to the final combination of vaccinations after booster doses

6. Variations in Immunity According to Combination of Vaccines

Significant differences were found in the mean IgG levels according to the type of vaccine received in the initial vaccination schedule in the group of nursing students that were given different types of vaccine (BioNTech/Pfizer and Oxford/Astra-Zeneca), as can be seen in Table 4A. The mean IgG values generated by the BioNTech/Pfizer vaccine are, on average, higher or remain higher at 6 months after full vaccination than among those that received the Oxford/Astra-Zeneca vaccine.

Type of vaccine in initial schedule	HCWs n (%); IgG 6 m vacc (Mean)	NS n (%); IgG 6 m vacc (Mean)
BioNTech/Pfizer Oxford/Astra-Z Moderna Statistic and p-value	137 (100); 3,041.46 AU/ml - - -	42 (52%); 2,859.86 AU/ml 35 (43%); 1,795.26 AU/ml 2 (2.5%); 6,688.30 AU/ml Mann Whitney U test= 435.00; p=0.002(**)
IgG mean values expressed in AU/ml – The comparison statistic and p-value are shown. (**) In the comparison of means, the two Moderna cases were discarded (n<5). Source: own preparation. Population from the ICM of Almansa (HCWs) and the Albacete UCLM Faculty of Nursing (NS).		

Table 4A. Acquired immunity in HCW and NS post-vaccination. Type of vaccines and IgG levels at 6 months after complete vaccination.

	Healthcare workers	Healthcare workers	Nursing students	Nursing students
Vaccination schedule	BioNTech/Pfizer + BioNTech/Pfizer	BioNTech/Pfizer + Moderna	Oxford/Astra-Zeneca + BioNTech/Pfizer	BioNTech/Pfizer + BioNTech/Pfizer
N (number of participants)	15	81	27	29
Mean IgG anti-SARS-CoV-2	27,802.98	26,447.05	9,051.44	25,205.42
CI for the mean (95%)	23,462.83 – 32,143.13	24,035.23 – 28,858.87	8,029.18 – 10,073.7	22,414.73 – 2,7996.21
SD	1,6809.32	21,706.43	5,311.81	15,028.84
Median	33,199.60	18,817.90	7,269.00	28,807.50
Interquartile range (IQR)	50,232.60	78,955.40	20,521.00	62,729.90
Statistical tests	Mann-Whitney U test= 548.0 p= 0.548 (non-significant differences)		Kruskal-Wallis test= 18.53 p< 0.001 (*)	
IgG mean and median values expressed in AU/ml. Vaccination schedules with <5 were discarded. The comparison statistic and p-value are shown. (*) Significant differences.				

Table 4B. Acquired immunity with booster dose. Combination of vaccines and IgG levels in vaccination population 6 months post booster

Table 4B shows the results of the anti-SARS-CoV-2 IgG measurements in participants after the booster dose, according to the different vaccine combination patterns. There are only three feasible vaccine combinations for statistical analysis.

Table 4B shows the IgG levels 6 months post booster dose, where it can be seen that the homologous combination with BioNTech/Pfizer (Pfizer + Pfizer) shows the highest IgG values. Table 4B also shows that the homologous vaccination (Pfizer + Pfizer) yields similar mean values to the heterologous vaccination (Pfizer + Moderna), as can be seen from the comparison of measurements in healthcare professionals that received this combination of vaccines, where there are no significant differences.

The variations in the mean IgG values according to the vaccine schedules can be seen in the measurement 6 months post-booster in the nursing student group: the IgG values of the students differ significantly depending on whether they received the AZ + Pfizer combination or Pfizer + Pfizer the combination. The mean values of IgG against SARS-CoV-2 are much higher on average with the homologous vaccination of the double dose with Pfizer, while in the Oxford/AstraZeneca combination, the mean antibody values are lower ($p<0.001$).

7. Adverse Effects of Booster Dose Vaccines

The side effects of the vaccines are mild and local in most cases, with little variation attributable to vaccine type. Table 5 shows the distribution of these side effects, by vaccine type in the booster dose.

The side effects of the third dose (booster) of the SARS-CoV-2 vaccines are mild and local symptoms are generally predominant. For example, local pain affects more than half of those who received BioNTech/Pfizer (52.6%) and 45.1% of those vaccinated with Moderna 82. Redness and local itching at the injection site are common after the booster dose and the only symptom presenting a significant difference in the proportion affected is pain in the limb (39.5% of those vaccinated with BioNTech/Pfizer and 17.1% of those given a dose of "Moderna 82".

General malaise, fever and chills are common general symptoms. General malaise is reported in more than a third of those who received booster doses; more than 20% had fever and between 9% and 22% had chills. Drowsiness and tiredness also affected 9.8% of those given Moderna and 14% of those vaccinated with BioNTech/Pfizer. No serious side effects were reported in either of the groups under study.

	Type of booster dose vaccine	
	BioNTech/Pfizer	**Moderna 82**
N (nº of participants)	71	98
Organ and system involvement: - Adverse reaction	Number of cases (n) and % of those vaccinated	Number of cases (n) and % of those vaccinated
Disorders of the blood and lymphatic system: -Lymphadenopathy	 5 (6.6%)	 7 (8.5%)
Immune system disorders: - Anaphylaxis - Hypersensitivity	0 0	0 0
Psychiatric disorders: - Insomnia	1 (1.3%)	2 (2.4%)
Nervous system disorders: - Dizziness - Facial paralysis - Drowsiness/ Tiredness - Paresthesias	 3 (3.9%) 0 11 (14.5%) 0	 0 0 8 (9.8%) 1 (1.2%)
Gastrointestinal disorders: - Nausea - Diarrhoea / Vomiting	 2 (2.6%) 3 (3.9%)	 5 (6.1%) 2 (2.4%)
Musculoskeletal and connective tissue disorders: - Limb pain (*) - Arthralgia - Myalgia	 30 (39.5%) 5 (6.6%) 9 (11.8)	 14 (17.1%) 8 (9.8%) 11 (13.4%)
General disorders and local disturbances at the injection site: - General malaise - Fever / Febrile Fever - Local pain - Fatigue - Chills / Shivers - Local swelling - Local redness - Itching at injection site	 29 (38.2%) 17 (22.4%) 40 (52.6%) 6 (7.9%) 7 (9.2%) 15 (19.7%) 7 (9.2%) 1 (1.3%)	 27 (32.9%) 18 (22%) 37 (45.1%) 5 (6.1%) 18 (22.0%) 15 (18.5%) 10 (12.5%) 2 (2.4%)
Skin and subcutaneous tissue disorders: - Hyperhidrosis - Generalised exanthema - Generalised itching	 1 (1.3%) 1 (1.3%) 0	 0 1 (1.3%) 0
Metabolic and nutritional disorders: - Decreased appetite	 3 (3.9%)	 0
Other (specify): - Menstrual disorders - Elevation of blood pressure - Headache	 0 0 0	 1 (1.3%) 1 (1.3%) 0
(*) Significant differences. Pearson's Chi-squared: 10.27; p= 0.006. Source: own preparation. Population from the ICM of Almansa (HCWs) and the Albacete UCLM Faculty of Nursing (NS).		

Table 5. Adverse effects after booster dose. Distribution by vaccine type

8. Discussion

Much work remains to be done to fully elucidate the characterisation of the human response to COVID-19 vaccines. It is thus of interest to study the humoral response and to decide whether booster doses and seasonal vaccination campaigns will serve to definitively control the pandemic [12]. This debate is still ongoing despite COVID-19 no longer being an international health emergency.

Since the appearance of the Delta variant of SARS-CoV-2, a large number of infections have been detected in healthcare workers. Therefore, vaccinating healthcare workers with a booster dose has been recommended due to the concern about these breakthrough infections, which would then continue to be more numerous with the Omicron variant, as reported in the study by Yang et al [18].

Despite the impact of the pandemic on HCWs' work performance and overall health, adherence to pandemic prevention measures is high. In our study, vaccination adherence was more than 95% for the first doses and over 93% for the booster dose. We found high adherence to the vaccination programme, although 2% of our participants delayed the first dose. Those who expressed doubts or refused the initial vaccination were typically hesitant, with the respondents' main concern being safety or possible side effects [19].

The effectiveness of the vaccines is demonstrated both by the level of IgG after vaccination and by comparing the incidence to assess the extent to which they prevent and protect against infection. Our data show that the percentage of individuals that were infected initially declined sharply (85% less) between 2020 and 6 months after full vaccination, but then increased notably in 2021 and 2022, to around 40% infected in 18 months [19,20]. The increase in infections in 2022 that support the evidence that the monovalent vaccine did not protect individuals from infection by other SARS-CoV-2 variants such as Omicron, which was prevalent that year. On the other hand, the differences in risk exposure in HCWs and NS are observed although the post-vaccination rate of infection was similar. It is possible that the lower occupational exposure rate and the restrictive measures in 2020 correspond to a lower infection rate in NS than in HCWs. However, after vaccination, the relaxation of isolation measures and the spread of the Delta and Omicron variants the sources of infection diversify and non-occupational exposure (family, contacts with friends) multiplies, such that in 2021-2022 infection rates are similar in both groups. In the same line, the study by Arashiro (2023) evaluates the efficacy of the vaccines in the period of the Delta and Omicron variants, quantifying the decreased incidence rate in vaccinated individuals. This decrease was 88% in the case of Delta 3 months after the second dose of vaccination, while during the period of Omicron the vaccine effectiveness fell to 56% after the second dose and to 52% 6 months after the second dose. Following the booster dose, however, vaccine efficacy rose to 74% [9].

Post-vaccination, antibody levels are positive at 6 months and rise considerably after the booster dose, remaining high 6 months later. In our findings, the first booster dose increases IgG by 590% in NS and 755% in HCWs, the two groups in our study, in line with other works. Differences in humoral response were different in the study populations at 6 months after the booster dose. We attribute these differences to the different vaccination schedules received by NS and HCW, in part as well as to differences in SARS-CoV-2 exposure throughout the study. Canetti et al. conducted a large prospective cohort study of healthcare workers and evaluated vaccine efficacy and humoral response after booster doses in a period when Omicron was the predominant variant in Israel. They concluded that a third dose of BNT162b2 vaccine led to an improved, sustained immune response compared to the results of IgG measurements after the second dose. Nonetheless, the additional immunological benefit of the fourth dose was much smaller and had completely diminished by 13 weeks post-administration [7].

The changes in immunity after booster doses provide clear evidence of increased IgG levels in the groups in our study. The variable effectiveness of COVID-19 vaccines across different demographic groups is a phenomenon corroborated by research [21,22]. The study by López Bernal et al. reveals that healthcare workers may have different efficacy rates compared to other segments of the population, thus underscoring the need to consider the specific characteristics of each population when designing vaccination strategies. We have not noticed differences in SARS-CoV-2 infection incidence on the basis of IgG titre, although we did find an association between hybrid immunity and higher IgG levels.

In assessing the efficacy of vaccines in generating immunogenicity, studies have addressed the differences in vaccination regimens and factors modifying the immune response, both in the initial schedule and in subsequent booster doses. The follow-up study by Moore et al [13]. on a cohort of healthcare workers who received different vaccination schedules concluded that humoral and cellular immune responses are well maintained over time - particularly in individuals with combined vaccine-induced and infection-induced immunity ("hybrid" immunity)- and may contribute to continued protection against severe disease [13].

Mojadadi et al., in their review of the efficacy of homologous and heterologous vaccines after the third dose, suggest that mRNA vaccines in a homologous regimen induce stronger antibody responses against SARS-CoV-2 compared to other vaccine schedules. In contrast, viral vector and inactivated vaccines show satisfactory immunogenicity in a heterologous regimen, especially in combination with mRNA vaccines [16]. Our results show that mRNA vaccines yield better antibody responses in both homologous (Pfizer + Pfizer) and heterologous (Pfizer + Moderna) regimens, which was evidenced by the comparison with a group of nursing students who received AstraZeneca (adenoviral vaccine), which generated significantly lower antibody responses after initial vaccination with AZ and after the heterologous regimen (AZ+Pfizer) in booster doses. Barros-Martins et al. (2023) bring attention to the challenges and call for further research to fully understand the long-term risks and benefits of these heterologous combinations. They highlight the positive immunogenicity, while also underscoring the importance of cautious evaluation prior to implementation [23].

Pani et al. (2022) examine the antibody response to COVID-19 booster vaccination. Their findings evidence universal efficacy in the study population, with a significant increase in antibody titres post-booster. Certain factors, such as gender, age and previous booster titres influenced the antibody response, although post-booster titres were similar in all subgroups. Their study corroborates the effectiveness of the BioNTech booster dose, regardless of the type of pre-booster vaccine [11].

The systematic review by Sadeghalvad et al. (2023) evaluates studies on the efficacy of vaccines in healthy individuals and in those with underlying diseases. Almost all the vaccines investigated were well tolerated and generated effective immune responses. The authors highlight the possibility of inducing higher antibody responses with longer intervals between doses. Although adverse effects were observed, vaccine efficacy prevailed [24].

In short, the existing research supports the idea that the effectiveness of COVID-19 vaccines varies across demographic groups, including healthcare workers. Additionally, vaccine combinations represent a promising strategy, although further research is needed to address outstanding questions regarding their long-term safety and efficacy. These findings underline the importance of tailoring vaccination strategies to maximise protection in specific groups and of actively adapting to the evolving epidemiological situation.

A cohort study conducted in Thailand reported no cases of severe COVID-19 with the third dose during the Delta period or with the fourth dose during the Omicron period. All the vaccine types used for the booster dose in Thailand boasted similar protection against severe COVID-19. The authors concluded that the booster doses provided a high level of protection against severe COVID-19 outcomes and that booster campaigns should focus on enhancing coverage, using all available vaccines to ensure optimal protection [25].

Regarding the factors associated with IgG levels after a booster dose, Padoan et al. (2022) assessed the post-booster decline in humoral immunity in individuals (healthcare workers) with homologous vaccination 3-4 months after administration, conducing that elevated IgG levels persist 3-4 months subsequent to the booster dose and that their decline is less pronounced over time in COVID+ individuals [26]. These data run counter to our results as regards IgG levels 6 months after the booster dose, but coincide on the associated factor, that is, a history of COVID-19 infection.
The study by Haveri (2023) compared the effect of the third dose of vaccine in healthcare workers and older adult population, both of whom were exposed to infection with the Omicron variant. Post-booster, healthcare workers showed high IgG concentrations, while levels were lower in the older adult population. The lower titres in frail older adults suggest inadequate protection against breakthrough Omicron infections although protection against severe COVID-19 is expected [27].

Other factors under study that may impact the immune response to the vaccine, such as gender, smoking or side effects of the vaccine, are not confirmed in all research. Older age groups and daily consumption of alcohol, however, were significantly associated with lower IgG levels in a study of healthcare workers (Ikezaki et al, 2022), leading the authors to propose additional vaccine doses for these risk categories [28]. Our findings show neither age nor risk habits (regular alcohol consumption or smoking) are associated with variations in IgG levels measured 6 months post-booster dose, despite smoking having been associated with lower IgG levels in previous measurements (6 months after full vaccination) [20].

The greater the time that elapses between vaccination and infection, the more immunity against COVID-19 appears to increase. This suggests that longer intervals between vaccine doses and booster doses may improve the immune response, as reported in the findings of Bates et al (2023): "Neutralizing titers were significantly improved for those with longer vaccination-infection intervals of up to 400 days, compared with those with shorter intervals". However, the authors state that further studies are needed to establish whether vaccines can elicit the same level of response and durability provided by hybrid immunity and suggest that the best strategy for long-term protection arguably involves the addition of alternative vaccine types that better mimic natural infection [29].

Concern about the adverse effects of the vaccines has been ongoing since the beginning of the SARS-CoV-2 vaccination schedule. Rashedi et al. already raised doubts about the efficacy and extent of side effects at the end of the first year of the availability of vaccines. Their study underlines that some undesirable effects of vaccinations had limited (and interrupted) vaccination in countries where not all types of vaccines were available. The authors also referred to the possibility of a strategy of mixing vaccines (a vaccination schedule with different types of vaccines) being a better way to deal with the problems of adverse effects, and there was already discussion at that time about heterologous vaccines in the first and second doses of the vaccination process [30].

Vaccine safety has been assessed by estimating the presence of adverse effects and showing the incidence of these after different doses. For example, a study by Copona Olmos in 2022 found adverse effects in immunised workers in 8.2% of cases at the first dose and 9.5% at the second dose [8]. In our study, the different adverse effects depending on the type of vaccine were primarily observed after the first and second doses of the vaccines, generating mild local and general effects in a large proportion of the population, with and with significant variations according to vaccine type [19].
Given the immunity provided by SARS-CoV-2 vaccines rapidly declines, doubts have recently been raised about their ability to prevent recently been questioned. Additionally, evidence is growing that, as with many other vaccines, they do not produce sterilising immunity, meaning individuals may suffer frequent reinfections. In addition, recent studies have found abnormally high levels of IgG4 in persons that were administered two or more injections of the mRNA vaccines. This has given rise to new concern that increased IgG4 synthesis resulting from repeated vaccination with mRNA with high concentrations of antigen may also cause autoimmune diseases, and foster the growth of cancer and autoimmune myocarditis in susceptible individuals, as reported by UbersKi et al. in a recent publication [31].

There is discussion in the scientific community as regards the relative efficacy of the vaccines available against the new variants of COVID-19 and the need to carefully select the groups to whom booster doses should be recommend. Future booster doses should be prudently programmed to coincide with waves of the virus or to be available seasonally, as is done with the influenza vaccine. It remains to be seen whether multivalent booster doses will result in increased durability of immunity, as suggested by Cannetti et al [7].

As is clear, the general consensus on the efficacy of the vaccines has shifted to accepting, with distinctions and doubts, their contribution to improving immune response and combating morbidity and mortality, without forgetting that the immunity acquired after exposure to the virus has prepared the population and limited the worst adverse effects of SARS-CoV-2 infection. The commercial interests of the multinational corporations that have created and commercialised the vaccines have conflicted with observational studies of post-vaccination efficacy, while certain aspects, such as the real preventive capacity of the vaccines against infections, severe evolutions and deaths, the efficacy of the vaccines against new variants, the limited duration of the immunity they provide, their adverse effects and the synergistic role of acquired immunity are under debate and need to be redefined, as Silva and Bloch report in a study on

the changing narratives about COVID-19 vaccines in 2021 and 2022 [32].

9. Limitations

The sample of students could not be completed by means of simple random sampling, and we were hence obliged to resort to a convenience sample from the study population that had shown interest in participating. Consequently, the sampling lacked equiprobability. Additionally, we did not achieve the sample size calculated for this group, and the inference of the results is thus limited. We must also note the limitations of this type of observational study, in which part of the data is self-reported, involving the possibility of information or memory biases in the reporting of symptoms, adverse effects or dates of positive tests. In this sense, a possible information bias could have occurred in the assessment of the incidence of COVID-19 as no screening tests were carried out in the healthcare workers or students as a whole, and therefore, asymptomatic or mildly clinical cases not detected by PCR or Ag-test could have been missed. The study has limitations as regards the generalizability of its results. This is because our population has a young and healthy adult population profile, which does not reproduce the full variability of subjects in terms of ethnicity, age or other conditions. Furthermore neutralizing IgG levels were not measured. Neutralizing antibodies have not been evalauted for technical reasons, namely, a restriction derived from the possibilities of the laboratoy that collaborated in the work (cited in the acknowledgements). Finally, 18.3% of the initial population was lost during, limiting the inference of the findings.

10. Conclusions

Following vaccination, antibody levels are positive at 6 months and rise considerably after the booster dose, remaining high 6 months later. The booster dose increases IgG levels by 590% to 755% in the two groups under study, namely, healthcare workers and nursing students.

The differences in IgG levels found after the booster dose, according to homologous or heterologous vaccination regimens, are statistically significant in the comparison of Pfizer+ Pfizer versus AstraZeneca + Pfizer. In contrast, no variations are observed between the homologous (Pfizer+ Pfizer) and heterologous (Pfizer+ Moderna) combinations.

The factor that marked a difference in the assessed level of IgG was prior SARS-CoV-2 infection (hybrid immunity). Another factor associated with lower IgG levels was smoking, which showed significant differences when evaluated after the initial vaccination, although this was not reflected in the post-booster measurements.

A high rate of both general and local mild adverse events was detected. Significant differences were revealed in these effects according to the types of vaccine received, in particular a high frequency of mild side effects (general and local) following vaccination with AstraZeneca in the initial vaccination schedule administered to the nursing students.

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