Evolution of acquired humoral immunity after full vaccination against SARS-CoV-2. IgG levels in healthcare workers at 6 and 9 months

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Abstract

Background

The COVID-19 pandemic continues to be a major worldwide health problem. The present study aims to contribute to surveillance of the immune and clinical response of vaccines to SARS-CoV-2.

Methods

Observational study of medication on acquired immunity and effectiveness of vaccines. Population: 620 workers in the health service of Almansa (Spain). Representative sample of 150 individuals. Sociodemographic, clinical, and epidemiological data and samples were recorded to determine anti- SARS-CoV2 serum IgG levels 6 and 9 months after vaccination with Pfizer.

Results

Mean age 46.45 years; 76% women; 85.1% working in a hospital. 19.3% had had COVID-19 in the year prior to vaccination. 96.7% were fully vaccinated with Pfizer/BioNTech. At 6 months, 100% seropositivity and mean IgG levels of 3017.2 AU/ml. Significant variations in IgG levels in individuals with prior COVID-19 infection and smokers. At 9 months, 99.3% remained seropositive; 2.8% infected after vaccination. The repeated measures analysis showed a difference in means of 669.0 AU/ml (significant decrease in IgG levels of 28.9%).

Conclusion

Antibody levels remained positive 6 and 9 months after vaccination, although IgG levels were found to decay.

1. Introduction

The COVID-19 pandemic, declared by the World Health Organization (WHO) in March 2020, continues to be a major problem with enormous impact. The fight against SARS-CoV-2 has focused the efforts of healthcare professionals and scientific researchers, with the aim of tackling the problem at all levels, from prevention, protecting the population by means of vaccines, to caring for cases (1)(2).

In December 2020, the first vaccines were authorized and began to be administered in various countries. According to the WHO, by 21 February 2022, a total of 10,407,359,583 doses had been administered (3). Vaccination coverage in Spain is high, with 93.0% of the population aged over 12 fully vaccinated and more 54%of the general population with booster doses (4).

Since its emergence, the SARS-CoV-2 virus has been constantly evolving. To date, the WHO has designated five variants as of concern, namely, Alpha, Beta, Gamma, Delta and Omicron, considering their impact in transmission, the severity of illness or their ability to evade immune protection (5) (6) (7).

Vaccine efficacy ranges from 50–95% (8). The long-term protection of antibodies against subsequent reinfection after COVID-19 and/or vaccination has not yet been fully established. Understanding of antibody kinetics against SARS-CoV-2 and its vaccines is evolving rapidly (9). Monitoring the immune response against SARS-CoV-2 is essential to evaluate long-term vaccine efficacy. Immunoglobulin G (IgG) antibodies constitute an appropriate tool to reach this goal, especially regarding the antibody trend induced by the new class of mRNA vaccines against SARS-CoV-2, which is still insufficiently defined (10).

The WHO suggests many pivotal questions remain about the effectiveness of vaccines in real-world settings, which can only be answered in studies on post-introduction vaccine effectiveness (11). Independent experts agree that knowledge about the protection provided by these vaccines will emerge in the coming months (12). It is worth noting that the length of the protection provided by the vaccines may vary, as shown by immunization against other diseases such as flu (13).

The TAG-CO-VAC (WHO Technical Advisory Group on COVID-19 Vaccine Composition) believes that vaccines against COVID-19 are necessary and should be developed and studies are needed to monitor the immune and clinical response of the vaccines (13). Questions remain unanswered as regards the duration of immunity and whether the new variants appearing will be neutralized by the antibodies generated by current vaccines (14). Vaccines against SARS-CoV-2 are highly efficient against severe forms of the disease, hospitalization and death. However, insufficient protection against several circulating viral variants could suggest a decrease in immunity and the need for additional vaccine doses (15). Many countries are administering a third dose of COVID-19 vaccines, but the evaluation of vaccine-induced immunity is insufficient(16).

Healthcare and socio-health professionals have been significantly affected by the COVID-19 pandemic. The report published by the Spanish Ministry of Health (10 February 2022) revealed 204,094 cases of infection since the onset of the pandemic (17). Spain has one of the largest percentages of infected healthcare employees, which justifies the prioritization of immunization in this population, with their being one of the first groups to be vaccinated (18). Healthcare workers (HCWs) were among the first group of people vaccinated (19). Characterization of the kinetics of antibody response to vaccination is important to devise future vaccination strategies and studies on workers in the healthcare sector have pioneered both the assessment of the occupational risk of COVID-19 and the surveillance of the immune and clinical responses to the vaccines administered to date.

2. Aims

To identify the sociodemographic, clinical, and epidemiological characteristics associated with occupational exposure to SARS-CoV-2 in a population of healthcare workers.

To determine seroprevalence and to measure levels of antibodies (IgG) against SARS- Cov-2 at 6 and 9 months after vaccination.

3. Methodology

3.1 Design

Prospective, longitudinal study. This is an observational medication study (OMs) on acquired immunity and effectiveness of SARS-CoV-2 vaccines.

3.2 Population

A total of 620 workers from the Integrated Care Management (ICM) of Almansa (Public Health Service of the region of Castilla-La Mancha, Spain). Sample size: was calculated with the objective of guaranteeing a precision of ± 3% at a 95% confidence interval, assuming a true seroconversion rate of 95%. We added an additional 10% to this number, in case of possible losses. The resulting sample size was 179 individuals. Simple random sampling (SRS) was used, and the persons selected were invited to participate in the study, using an informed consent form. Those that accepted were included in the sample. Those that failed to answer or refused to participate were replaced by others from the same population. The final sample of persons recruited and that participated in the study comprised 150 individuals (83.8% of the initial sample).

3.3. Study variables

The main study variable was the immunological response to the vaccine, measured according to the level of IgG antibodies 6 and 9 months after full vaccination. The following were also studied: sociodemographic variables, such as age, sex, professional category, job and healthcare facility; clinical and epidemiological variables, such as antecedent of COVID19 and date of PCR, occupational exposure to COVID19, risk factors and level of risk (Ministry of Health Classification), date and type of vaccination and prior COVID19 diagnosis if applicable.

3.4. Data collection:

An internally developed questionnaire on sociodemographic, clinical, epidemiological data, date of vaccination and other information of interest.

Determination of anti-SARS-CoV2 serum IgG levels: the AlinitySARS-CoV-2 IgG II Quant assay (Abbott®) was performed for all the samples. This test is based on chemiluminescent microparticle analysis (CMIA), which quantitatively and qualitatively determines IgG antibodies against the receptor binding domain (RBD) and spike protein subunit 1 (S1) of SARS-CoV-2. According to the laboratory, sensitivity is 100% and specificity 99.9%. The unit of measurement is AU/ml (arbitrary units per milliliter) (20)(21). Serum samples were centrifuged at 2500 rpm for 10 minutes and stored at 4ºC until processing.

3.5. Data collection procedure and samples:

The eligible population was given a study information sheet and an informed consent form. The data collection questionnaire was anonymized. Participants were called to attend serological analysis on two dates approximately 6 and 9 months after full vaccination, having previously completed the questionnaire.

3.6. Follow-up and control of possible losses:

Participants were followed up and given an appointment for the second sample (November-December 2021). This second data collection involved various noteworthy events since the previous measurement, from both a clinical viewpoint and an occupational exposure perspective. Despite the personal contact with the participants in the second sampling, not all the individuals from the first measurement were available and/or met the requirements, and thus the comparative analyses of IgG evolution were limited to 132 persons (9 months).

3.7. Statistical analysis:

The data were processed and analyzed using SPSS® IBM 24.0, which was also used for the statistical analysis.

We conducted a univariate descriptive analysis using central tendency and dispersion measures: arithmetic means, standard deviations (SDs), minimum and maximum, for the continuous variables, and absolute frequencies and proportions for the categorical variables. Confidence intervals were calculated at 95%. 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 (chi-square tests, Student's t test, ANOVA…). The relationships between the quantitative variables were analyzed using Spearman's correlation.

In all cases, bilateral comparisons were used with a significance level of $p < 0.05$.

3.8. Bias control

To minimize losses, contact was maintained with all the participants. Those who wished to know their results were duly informed, individually and upholding confidentiality in all cases.

3.9. Ethical considerations.

The project was approved by the Clinical Research Ethics Committee of the University of Castilla-La Mancha (UCLM) and the Albacete Health Service Area, as well as by the Spanish Medicines Agency (5/21/2021). The Castilla-La Mancha Health Service (SESCAM) gave its approval to the study (Code 2021-27) on June 11, 2021. It was published in the Spanish Registry of Clinical Trials, which is mandatory for this type of design: observational study of drugs. All the participants gave their signed informed consent to participate in the study. Recommendations about personal data processing followed current Spanish legislation. All methods were performed in accordance with the relevant guidelines and regulations. The authors declare they have no conflicts of interest.

4. Results

Below, we present the results of the study, which respond to our aims. The sociodemographic characteristics of the study population are: mean age 46.45 years; (SD = 9.95); Range = 41.74; Minimum value = 23.9 years; Maximum value = 65.8 years; Median (Mn) = 45.4 (9 subjects did not report their age). Table 1 shows distribution by age group, sex, education level, occupation, and area of work (hospital, primary care and socio-health care). There is a notably high proportion of women, 40 to 49 years is the largest age group (42.6% of total), a majority have university studies and a large percentage are nursing professionals. Thus, the predominant profile is that of a female nurse working in a hospital and aged below 50.

4.1. COVID-19 incidence rate in the study population.

The cases diagnosed before vaccination were recorded and the cumulative incidence rate (CIR) was calculated, summing both the cases with a PCR diagnosis and suspected and possible cases (according to the current classification (1) (22). COVID-19 incidence in the year prior to vaccination was 29 cases in the study sample, which represents a rate of 19.33%. In most cases, the origin of the source of infection was unknown, while a third of cases reported work-related origins. Subsequent to vaccination, 7 cases were reported, 4 after full vaccination and 3 after the first vaccine dose.

4.2. Factors related to immunity.

The distribution in the population of health habits related to immunity, such as the consumption of toxic substances (tobacco and alcohol), was evaluated (Table S1 in supplementary material). It is worth highlighting the percentage of active smokers (17.5%), which is a lower proportion than the most recent data on Spanish adult population. Alcohol consumption is more widespread, although the frequency and weekly consumption declared by participants is moderate to low, as only 12.8% report weekly consumption, which does not reach the risk-level consumption recognized by the WHO.

We found high adherence to vaccination in both the healthcare and non-healthcare staff, with only 2.7% of unvaccinated individuals at the start of the study. Nonetheless, most of the latter had delayed the vaccination and, before the end of 2021, three of every four unvaccinated participants had initiated the vaccination process.

4.3. IgG levels at 6 months. Quantification and analysis according to immunity-related variables.

All the 142 vaccinated participants had a positive antibody (IgG) level 6 months after full vaccination, considered as $\geq$ 50 AU/ml. Table 2 shows the descriptive statistics for IgG levels 6 months following full vaccination and the bivariate analysis with factors that might cause variations in immunity. Among the variables typically considered as affecting IgG levels, an association was found between smoking (as an immune response depressor) and the antecedent of SARS-CoV-2 infection as an inductor of a higher immune response (hybrid immunity). No association was found between IgG levels and age, occupational exposure to COVID-19, type of occupation or moderate alcohol consumption.

Table 2
Descriptive statistics for IgG levels in vaccinated population. Bivariate analysis was performed with Log 10 of IgG ang geometric means.

	IgG 6 months AU/ml	IgG 9 months AU/ml
Total individuals (n)	137	132
Mean	3,017.2	2,941.3
(95% CI)	(2105.4-3928.9)	(1901.6–3981)
Geometric mean	1,402.66 AU/ml	1,053.69 AU/ml
SD	5,396.6	6,038.3
Minimum value	62.6	< 50
Maximum value	36,644.7	40,178.4
Median	1,158	840
Interquartile range	571.9-2,585	399.4-2,485.3
Sex	**Geometric mean comparison**	
Women	1,285.28	981.9
Men	1,827.83	1,299.15
Statistic and p-value	t = 1.556; p = 0.122 (NS)	t = 1.053; p = 0.295 (NS)
Age groups		
< 35 years	2,006.47	1,783.36
35–49 years	1,165.80	987.6
≥ 50 years	1,647.95	972.63
Statistic and p-value	ANOVA, Dunnett test. p > 0.05	ANOVA, Dunnett test. P > 0.05
Smoking		
Non-smokers	1,787.83	1,209.41
Smokers	783.38	640.23
Ex-smokers	1.364.61	1.160,16
Statistic and p-value	ANOVA, Dunnett test. p = 0.005*	ANOVA, Dunnett test. p > 0.05
**Smoker and non-smoker comparison shows significant differences*		
Occupational exposure to COVID-19		
Yes	1,234.33	910.59
No	2,270.78	1,759.77
Statistic and p-value	t = 1.783; p = 0.087	t = 1.732; p = 0.096
Prior COVID-19 infection (prevaccination)		
Yes	3,893.11	3,104.72
No	1,021.50	777.34
Statistic and p-value	t = 6.649; p = 0.000*	t = 5.591; p = 0.000*

IgG values expressed in AU/ml – The comparison statistic and p-value are shown. Data on study population from ICM Almansa (Albacete) 2022.

4.4. IgG levels and their evolution: follow-up at 9 months

Table 2 shows the descriptive statistics for IgG levels 9 months after completing full vaccination. In only one case was the antibody level negative (< 50 AU/ml), being a person with a low antibody level at the initial measurement (6 months). Seronegativity was 0.7%, with 99.3% retaining antibodies.

The most noteworthy result of the comparison between IgG values 6 and 9 months after full vaccination, in the complete sample, is a slight decrease in mean values. However, the differences are not statistically significant, as can be seen in the confidence intervals of the mean, as common data are included.

Figure S1 (supplementary material) shows the differences in the mean IgG values by sex and age group. These differences were not statistically significant.

In the repeated measures comparison of IgG levels (n = 102), the means difference, statistically significant, is 669.0 AU/ml, representing a fall in IgG levels of 28.9% (Table 3).

Table 3 shows the means differences between 6 and 9 month follow-up in groups where the results are significant or more pronounced than in the overall study population, as is the case of over 50-year-olds, men and smokers.

Figure 1 shows the distribution of IgG levels at 6 and 9 months for the individuals with two measurements.

(Insert Fig. 1)

Table 3
IgG levels in vaccinated population 6 and 9 months after vaccination. Means comparison (repeated measures).

Means comparison for IgG at 6 and 9 months (n = 102)	**Means, statistic and p-value**
IgG 6 months	2,368.52 (DE 2933.12)
IgG 9 months	1,699.48 (DE 2443.77)
	Wilcoxon Z= -7,770; p = 0,000
Groups in which the decrease in IgG levels is significant	**Means, statistic and p-value**
> 50 years:	1,971.63 (SD 2021.07)
IgG 6 months	1,241.99 (SD 1500.62)
IgG 9 months	Wilcoxon Z= -4.869; p = 0.000
Men:	3,345.23 (SD 3543.07)
IgG 6 months	2,653.07 (SD 2978.28)
IgG 9 months	Wilcoxon Z=-3.70; p = 0.000
Women:	1,998.96 (SD 2599.17)
IgG 6 months	1,338.67 (SD 2121.59)
IgG 9 months	Wilcoxon Z=-6.89; p = 0.000
Active smokers: IgG 6 months	1,151.94 (SD 1204.47)
IgG 9 months	673.18 (SD 626.10)
Non-smokers:	2,737.76 (SD 3507.67)
IgG 6 months	2,056.28 (SD 2961.99)
IgG 9 months	

Mean IgG values expressed in AU/ml – The comparison statistic and p-value are shown. Source: study population from ICM Almansa (Albacete) 2022

4.5. COVID-19 incidence rate between doses and after full vaccination.

In the study population, there were 7 cases of COVID-19 following vaccination, 4 after full vaccination and 3 after the first dose, yielding a CIR of 2.8%. The comparison of CIR for our healthcare workers before vaccination (CIR unvacc) and after full vaccination (CIR vacc) was CIR unvacc/CIR vacc = 6.88. In other words, COVID-19 incidence fell almost seven times less after vaccination, which represents an 85.46% fall in incidence rate, which can be attributed to the vaccination. Additionally, none of the cases diagnosed after vaccination presented either moderate or serious symptoms.

5. Discussion

Numerous studies have measured the immune response in healthcare workers to both SARS-CoV-2 infection and vaccines. Drawing on this bibliography, we compared our data with those from studies focused on similar variables (those assessing humoral immune response following vaccination with Pfizer and with follow-ups at several months). The healthcare professionals included in other studies have similar profiles to those of our sample, with an average age of 54.4 years and a mean of 46.3. The mean ages typically range between 40 and 48 (23) (24) (25), although some studies use a younger population with a mean age of between 33 and 37 (26), (27) and (28). In all cases, there is a greater proportion of woman, ranging from just over 50% (26), around 60–69% (27),(24), (25) and more than 70% (28), as in our study, with 76% of women. Some of the works include populations of which more than 80% are women (23), (29) and (30). The findings on age-related variations in the immune response are conditioned by this limited diversity in the ages of the health service employees. As regards the sex-related variations, given the largely female populations and the scant number of men in some groups, the differences reported are, arguably, not always significant or clear.

The incidence of previous exposure to SARS-CoV-2 in healthcare workers is typically higher than among the general population in their respective countries, which is in line with the results of our study. The incidence data vary greatly and tend to be reported as a percentage of persons infected, ranging between 7% and 25%. The range of values is as follows: studies with the lowest incidence rates of 7% and 7.8% (27) (30) (31); around 10% (32); others with figures similar to our 19.3%, such as 19.22% (24); and others with higher incidence rates of 23% (14) 25% (26) and 32,1% (33).

Based on a cutoff point of 50 AU/mL for IgG Spike positivity (RBD), 100% of the participants in our study had antibodies following vaccination, in line with other studies (14)(30). These results are described in some studies as seroconversion, as they present data compared with baseline levels, finding that all vaccinated employees without prior COVID-19 infection have positive IgG levels (9), or percentages close to 100% (30)(31)(32)(36). Discrepant data were also reported with 22.9%, being seronegative (19).

Terpos et al. followed up a group of healthcare workers for several months after vaccination, finding persistent but attenuated anti-SARS-CoV-2 humoral immunity at 3 months after second vaccination with BNT162b2 in healthy individuals (34) (32). Several studies have established peak level circulating antibodies at 3–4 weeks after the second vaccination, and there is a considerable consensus on the decrease in levels over time, especially after the third month (26). Additionally, most of the studies presents dispersion in the values in coherence with our findings.

The study by Rode et al. reported that most of the participants present positive values, from 50 to 2000 AU/mL was the most representative range of antibody titers in almost 80% of subjects (35). The interquartile range for our data at 6 months was slightly higher although the conclusions of immune response efficacy are mostly similar.

Among the factors related to differences in IgG levels on which there is more evidence, the most notable are age and previous infection. Regarding age, evidence suggests that younger individuals tend to present higher levels of immunoglobulin G anti-SARS-CoV-2 (19, 29 y 30). In our work, no association was found between IgG level and age.

We found no differences in IgG levels by sex, which is consistent with the findings of other studies (35 and 39). However, some studies have detected higher IgG levels in women in the initial immune responses after vaccination (9, 19, 21, 30, 32 and 34).

Comparisons of antibody levels in persons with previous infection and subsequent vaccination confirm hybrid immunity is more robust in IgG measurements a few weeks after vaccination. Individuals with prior infection present higher IgG levels at all time

points (31) and these differences are maintained various months after vaccination (9)(25)(35), although they are more pronounced in the early weeks post-vaccination. Seropositivity was significantly higher in healthcare workers with prior COVID-19 infection, according to the cross-sectional study by El-Ghitany et al. (33). In our study, the differences in IgG levels remained significantly higher in persons with prior infection both 6 and 9 months after vaccination.

Hansen et al. found that a single dose of the BNT1622b vaccine induces a robust antibody response in individuals with previous infection and that, in immunogenicity, is equivalent to a double dose of the vaccine (30). Consequently, it is considered that a single dose might be sufficient in individuals with previous infection, regardless of the time elapsed since the diagnosis (24). Nonetheless, a generalized decline in antibody levels over time has been found in vaccinated individuals both with and without prior infection.

Some works have found an impact on immune response levels of other factors, such as chronic diseases, smoking and high BMI. Nonetheless, the findings are inconclusive (9)(26). El-Ghitany et al. found a relationship with smoking, with antibody positivity being significantly lower in smokers (61.9%) compared to non-smokers (87.7%) ($p = 0.003$) (33). Our study also confirms that smoking inhibits immune response

As regards BMI, the studies by Hansen et al. (30) and de El-Ghitany et al. provide no conclusive data to support a relationship between high BMI and impaired immune response (33). Papadopoulos et al., however, found an association between older participants, higher BMI and the presence of autoimmune diseases with negative effects on the development of anti-SARS-CoV-2 antibodies 9 months after full-vaccination (36).

Our findings point to durable immunity despite the decline in antibody levels 9 months post-vaccination. Other works coincide with our findings. Studies indicate a decline from peak levels in neutralizing antibody titers, although these remained detectable in most participants 6 months post-vaccination (29) (37)(38). The data reported by Rode et al. at 6 months after full vaccination (mean IgG 966.0 AU/mL) are similar to those in our study (35).

The recent systematic review by Notarte et al., characterizing the kinetics of anti-SARS-CoV-2 antibodies following the second dose of a primary cycle of mRNA vaccination, revealed that the peak humoral response was reached at 21–28 days after the second dose. Subsequently, serum levels progressively decreased at 4–6 months post-vaccination. and the results showed that, regardless of age, sex, serostatus and presence of comorbidities, there is an antibody decay (39).

The studies that offer findings more than 6 months after vaccination (in general they do not exceed 8 or 9 months) show antibodies largely remain active, despite a notable decline in levels (37) (40), with important variations according to groups of subjects (41).

There is concern about whether the vaccines will be less effective against the new variants of the virus (Delta, Omicron, Omicron B.A.2). Although studies have already been published on Omicron and the Delta and Beta variants and RNA vaccines are reported to provide protection against severe and lethal forms of COVID-19 but infection persists against these SARS-CoV-2 variants (37) (41).

There is considerable agreement on implementing booster doses in high-risk population, such as daily alcohol drinkers, frail elderly, smokers and other groups, in whom both a greatly diminished humoral and cellular immune response has been evidenced, and in which booster doses may be warranted (16) (41) (42). Some studies point towards the need to customize additional booster doses to achieve an adequate neutralizing response against the new circulating variants, which justifies the decisions on the third dose implemented in European countries and those that may be recommended by health authorities in the coming months (34).

Immunosurveillance studies estimate the duration of immunity and are especially necessary for designing public health responses to the general population, healthcare workers and, particularly, specific population groups with a compromised immune response (43) (44). It is even noted that the heterogeneity of responses to vaccines suggests that personalized recommendations based on COVID-19 history and lifestyle are necessary (45).

6. Conclusions

The incidence of COVID-19 disease in healthcare workers is higher than in the general population.

A high proportion, close to 100% of immunized individuals was detected, with positive IgG levels at both 6 and 9 months after vaccination.

The immune response was found to be more robust in certain groups of individuals, with evidence of a clear, positive association with prior COVID-19 infection in vaccinated persons.

Non-smokers develop a more powerful immune response and present higher IgG levels compared to smokers.

Antibody levels remain positive 9 months after full vaccination despite the evidence of a decline in IgG levels.

7. Limitations

The external validity of the study might be limited by the mean age of the study population, given that it did not include individuals at age extremes. Additionally, as a healthy adult population, comorbidity was low.

Prior COVID-19 infection was self-reported by the participants. In the initial questionnaire and at the second sample taking, the participants reported any prior infection, providing data on the date of the diagnostic test (PCR o antigen test).

Data and sample collection was affected by circumstances beyond the control of the researchers, primarily changes in the employment status of the workers or a lack of response despite having agreed to participate in the study.

Between the first data and sample collection and the second, 18 subjects (12%) were lost, which may increase the error in the estimates.

The diagnostic method used to detect anti- SARS-Cov2 serum levels did not allow the neutralizing capacity of these antibodies to be determined. Additionally, we were unable to differentiate the antibodies generated by the vaccine (anti-S, RBD), from those generated naturally by participants following infection (anti-N assays).

Declarations

Data availability:

"The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request."

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Tables

Table 1 is available in the Supplementary Files section.

Figures

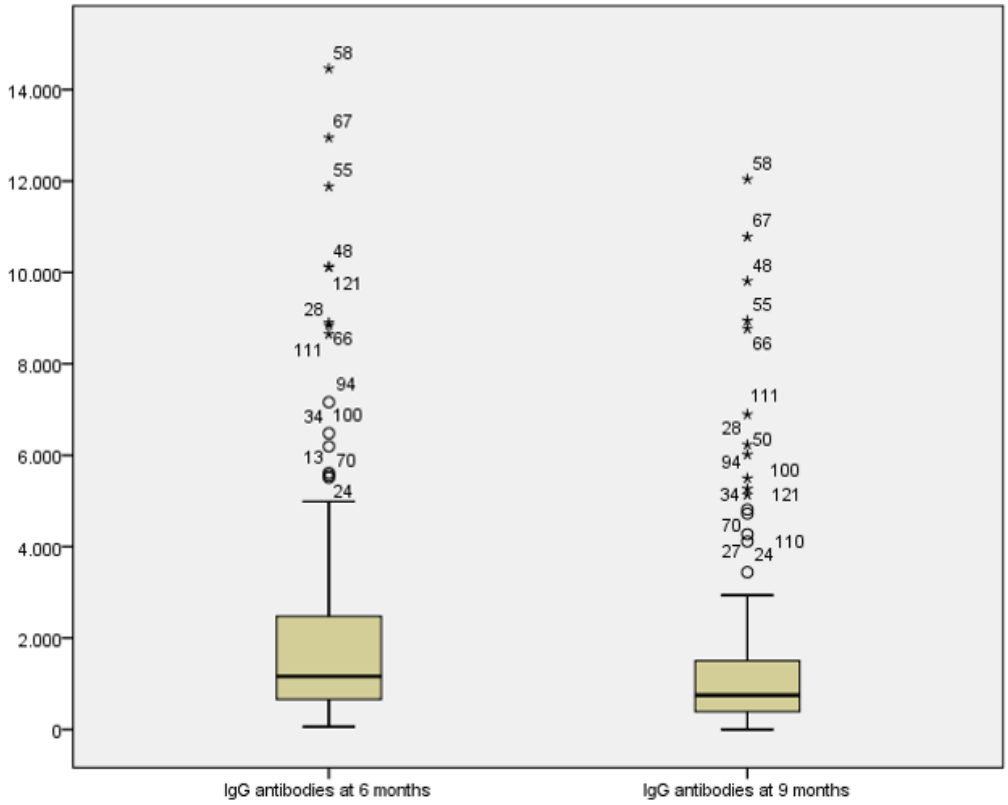

Figure 1

Distribution of IgG levels at 6 and 9 months

Supplementary Files

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- SuplementarymaterialLS.docx
- Table1.docx