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Seroprevalence of SARS-COV-2 Antibodies Associated with the Use of Personal Protective Equipment in Healthcare Workers

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ABSTRACT

Background: Hypothetically, PPE played an estimated influential role in preventing SARS-CoV-2 transmission among HCWs before immune-prophylaxis by vaccination in BH. Objective: This research aims to determine the relationship between PPE use and the serological response to SARS-COV-2 among HCWs. **Methods:** The sample contained 127 COVID-19 outpatients with an average age of 43.5 ± 10.8, 66% women, and 80 (63%) health workers as the study group (37% non-medical workers as a control subjects). The created questionnaire collected sociodemographic data on comorbidity or not, application of PPE, and severity of the clinical picture of COVID-19 infection. **Results:** All subjects were monitored for the dynamics of antibodies, separately for IgM- and Ig-G three times, repeated every 3 months (only three before immuno-prophylaxis by vaccinations). A serological investigation of subjects' blood was collected by trained medical staff in vacutainers with a clotting activator to obtain the subjects' serum centrifuged, separated, and tested on the AFIAS 6 COVID-19 apparatus. Healthcare workers who did not wear goggles had significantly higher IgM antibody levels than HCWs who use them [F=9.359 (1, 102), p=.003, partial η2=.084]. Also, HCWs who did not use a visor had significantly higher IgM nucleocapsid antibody titer than those who used it daily or occasionally [F=4.790 (1, 102), p=.031, partial η2=.045]. Increase SARAS-COV-2 IgM titer three, six, or nine months after past COVID-19 infection and before vaccination. **Conclusion:** It presents a new acute or recent asymptomatic infection in our HCWs and unrecognized COVID-19. This implies considerable source and unrecognized risk of transmission of the SARS-COV-2 virus, and among HCWs, COVID-19 is an infectious disease with a high prevalence rate.

Keywords: seroprevalence, SARS-COV-2, COVID-19, healthcare workers, IgM and IgG.

1. BACKGROUND

At the end of December 2019, a new, unknown respiratory infection in Wuhan, China, spread around the world at lightning speed in pandemic proportions and caused death (1-4). There was an urgent need to identify the causative agent and potential ways of its transmission and to take appropriate preventive measures, activities, and means. Pandemic threats such as SARS-COV2 resource-constrained settings where drugs and vaccines are unavailable, public health measures have been the only viable option in delaying pandemic development. For the prevention of SARS-COV-19, governments and state institutions were responsible for health and civil protection globally, including Bosnia and Herzegovina (BH) (5). Their fundamental purpose was not to prevent or stop the pandemic but to slow the transmission of the SARS-COV2 virus, that is, to reduce transmission, the prevalence of infections, and disease and death (6). In a very short time, the International Community for Taxonomy of Viruses (ICTV) identified a virus that belongs to β-coronaviruses and is very similar to SARS-virus and was named SARS-CoV2 (7). At that time, all SARS-COV-2 modes were

not known so the World Health Organization (WHO) recommended personal protective equipment (PPE) used for all healthcare workers (HCWs) to stop droplet transmission of the virus in close contact with asymptomatic and infected patients (8-9). Following this activity, Public Health at the national and international levels has extended the WHO recommendations to all employees and citizens who are not isolated from contact with other people for protection against airborne infections (10).

2. OBJECTIVE

This research aims to determine the relationship between the application of PPE and the serological response to SARS-COV-2 among HCWs.

3. MATERIAL AND METHODS

Study design and setting

The research was conducted as a longitudinal cohort study within the screening-mass testing of COVID-19 infection among HCWs from May 1, 2020. to December 2021. in the Cantonal Hospital "Dr. Irfan Ljubljankić" in Bihać. The screening was to determine the amount of anti-SARS-CoV-2 anti-nucleocapsid antibodies in examiners exposed to the SARS-CoV-2 virus the first three times of five every three months of serological measurements at a three-time interval (three-time points sub-cohorts).

Eligibility criteria

The sample contained 127 COVID-19 outpatients with an average age of 43.5 ± 10.8, 66% women, and 63% health workers with an average age of 52.5 ± 13.8, 66% (37% non-medical workers). All subjects were monitored for the dynamics of antibodies, separately for IgM- and Ig-G at three times, repeated every 3 months (only three before immuno-prophylaxis by vaccinations). According to the frequency of use of PPE, they have formed two or three sub-cohorts (respirator mask, visor, goggles, suit or gown (answer yes) and those who did sometimes or did not use PPE. Both healthcare and non-medical workers have used surgical masks and disposable surgical gloves.

Data collection

A questionnaire was created to collect sociodemographic data (age and sex), occupation (healthcare workers, non-medical workers), comorbidity or not, application of PPE, and severity of clinical picture of COVID-19 infection.

Outcome measures

A serological investigation of subjects' blood was collected by trained medical staff in vacutainers with a clotting activator to obtain the subjects' serum centrifuged, separated, tested (on the AFIAS 6 apparatus, AFIAS COVID-19), and frozen the rest of the serum at -20°C (11) for automatic qualitative and semiquantitative determination of SARS CoV2 specific antibodies (12). The antibody number in units of the cut-off COI index was presented. The COI for a negative result of IgG- and IgM- antibodies is < 0.9, and a positive is ≥ 1.1. An indeterminate result requiring retesting is between 0.9 and < 1.1 COI. The operating range is 0-200 COI (11).

Ethical principles

Each respondent received a verbal and written explanation of the research before voluntarily agreeing to participate in this study, and they had to give written consent to participate in the research. Respondents could withdraw at any time, and the confidentiality of their data was guaranteed. The study began with the prior approval of the Ethics Committee of the Cantonal Hospital "Dr. Irfan Ljubljankić" Bihać, number 01-1-33-3522/20. This study was conducted according to the ethical principles of the Declaration of Helsinki and legal regulations on biological material - patient blood.

Statistical analysis

The descriptive statistic parameters were means, standard deviations, or relative numbers and percentages for categorical data. Inferential statistics included repeated measures ANOVA and Kaplan-Meier. The Kolmogorov-Smirnov test was assessed data distribution according to the perceived results. Correlations were determined between the corresponding variables, depending on the structure of the obtained/analyzed data and the size of the analyzed samples. Also, a comparison was made to determine the differences between the observed samples and their measurement values. The antibody numbers were remeasured three times, and ANOVA analysis of variance was applied. Statistical significance was at p<.05. The above statistical analyses were performed using IBM SPSS, Statistics for Windows, version 21.0, Armonk, NY, USA.

4. RESULTS

Demographics, baseline characteristics, PPE data, and seroprevalence of anti-nucleocapsid antibodies IgM and IgG

Table 1 shows the demographic data of 127 COVID-19 outpatients divided into two groups according to their professional activity: healthcare workers HCWs (average age of 52.5, 75% women), and non-medical workers (average age of 43.6, women 47%) according to the study objective of wearing PPE and seroprevalence IgM and IgG antibodies. T We found significant differences by gender between these two groups (p<.001). There are no significant differences between the two groups by age, the severity of the clinical- picture of COVID-19 (78.8%: 74.5% with mild clinical picture), and the comorbidity. In both groups, the high sensitivity to SARS-COV-2 transmission in ages 40 to 49 is observed before vaccination. There is no statistically significant difference in continuous, daily wearing of surgical masks between healthcare and non-medical workers (99%: 87%, p=0.123). Latex gloves were worn significantly more frequently by healthcare workers than by non-healthcare workers (73%: 26%, p<.001). Specific healthcare PPE against SARS-COV-2 transmission through ambient air was monitored in two or three subgroups of healthcare workers (who applied PPE continued or sometimes or never). There were significant differences in these subgroups for all specific

Outpatient characteristics		Health care workers No=80 N (63%)	Non-medical workers No=47 N (37%)	p-value (χ^2-test)
Sex				
	Male	18 (22.50)	25 (53.19)	.001
	Female	62 (77.50)	22 (46. 81)	(12. 45)*
Age (subgroups, years)				
	20-29	7 (8.75)	2 (4.25)	
	30-39	14 (17.50)	16 (34.05)	.214
	40-49	35 (43.75)	18 (38.30)	(5.136)
	50-59	15 (18.75)	6 (12.77)	
	>60	9 (11.25)	5 (10.63)	
Severity of clinical picture				
	asymptomatic	4 (5.00)	3 (6.38)	.888
	mild	63 (78.75)	35 (74.47)	(0.237)
	moderate	13 (16.25)	9 (19.15)	
Have comorbidities				
	yes	46 (57.50)	31 (65.96)	.887
	no	34 (42.50)	16 (34.04)	(0.226)
Protective personal equipment				
Apply PPE**				
	every day	64 (80.00)	42 (89.36)	.356
	sometimes	11 (13.75)	4 (8.51)	(2.064)
	never	5 (6.25)	1 (2.13)	
PPE				
	every day	70	37	.423
	sometimes	9	9	(1.719)
	never	1	1	
Surgical mask				
	every day	79 (98.75)	41 (87.23)	.123
	sometimes	1 (1.25)	6 (12.77)	2.380
Respiratory mask				
	every day	12 (15.00)	1 (2.13)	.017
	never	68 (85.00)	46 (97.87)	(5.339)
Goggles				
	every day	18 (22.50)	2 (4.26)	
	sometimes	19 (23.75)	0	.001
	never	43 (53.75)	45 (95.74)	(24.956)
Visor				
	every day	25 (31.25)	1 (2.13)	.001
	sometimes	26 (32.50)	2 (4.25)	(39.929)
	never	29 (36.25)	44 (93.62)	
Gown				
	every day	31 (38.75)	0	.001
	sometimes	18 (22.50)	0	(46. 872)
	never	31 (38.75)	47 (100)	
Gloves				
	every day	58 (72.50)	12 (25.53)	.001
	sometimes	16 (20.00)	19 (40.43)	(28.372)
	never	6 (7.50)	16 (34.04)	
IgM1 seroprevalence		Health Care Workers No=70	Non-medical workers No=57	
	undetermined (re-test)	30 (42.79)	9(15.78)	
	negative	13 (18.61)	12(21.01)	.213
	COI positive	27 (38.60)	36(63.21)	(92.951)
IgM2 seroprevalence		No=68	No=50	
	undetermined (re-test)	39 (56.48)	19(38.00)	
	negative	18 (26.11)	10(20.00)	.116
	positive	12 (17.41)	21(42.22)	(73.334)
IgM3 seroprevalence		No=67	No=47	
	undetermined (re-test)	46 (68.71)	18(38.28)	

	negative	12 (17.88)	14(29.81)	.633
	COI positive	9 (13.41)	15(31.91)	(66.066)
IgG1 seroprevalence		No=70	No=57	
	undetermined (re-test)	4 (5.67)	5(8.83)	
	negative	1 (1.41)	0(00.00)	.430
	COI positive	65 (92.92)	52(91.17)	(118.017)
IgG2 seroprevalence		No=69	No=50	
	undetermined (re-test)	1 4 (20.27)	1(2.00)	
	negative	4 (5.31)	1(2.00)	.108
	COI positive	51 (73.92)	48 (96.00)	(123.227)
IgG3 seroprevalence		No=67	No=47	
	undetermined (re-test)	11 (16.38)	2(4.25)	
	negative	2 (3.00)	2(4.25)	.108
	COI positive	54 (80.62)	43 (91.50)	(123.227)

Table 1. Differences in demographics, type of COVID-19 clinical picture, comorbidities, PPE application, seroprevalences of IgM- and IgG-SARS-COV-2 between groups (HCWs and non-medical workers) during three repeated measuring and subgroups (healthcare workers)

PPE: respiratory mask (p=.017), goggles (p<.001), visor (p<.001), and mantel or protective clothing (p<.001). However, HCWs who consistently used latex gloves had significantly higher IgM anti-nucleocapsid antibody levels than non-medical workers (P=.001) (data not shown). A respiratory mask was never used by 85%, goggles by 54%, a visor by 36%, and gloves by HCWs by 7.5% of HCWs. Positive COI for IgM in HCWs and dynamics of seroprevalence in all three repeated measurements was 38.6% for IgM1, 17.41% for IgM2, and 13.41% for IgM3. Higher levels of COI seroprevalence for IgM in non-medicine workers were found: 63.2% for igM1, 42.2% for IgM2, and 31.91 for IgM3. A positive COI prevalence for IgG in HCWs in the first point has been confirmed: 92.9% for IgG1 with a slight decrease for IgG2 and IgG3.

Figure 1 linear graphs show IgM antibodies among the six subgroups of HCW during the three times assessed repeated every three months (three points of measuring). Healthcare workers who did not wear goggles had significantly higher IgM antibody levels than HCWs who use them [F=9.359 (1, 102), p=.003, partial η2=.084]. Also, HCWs who did not use a visor at all had significantly higher IgM anti-nucleocapsid antibody levels than those who used it daily or occasionally [F=4.790 (1, 102), p=.031, partial η2=.045]. Although IgM anti-nucleocapsid antibody levels were higher in HCWs who did not use protective gowns, there was no significant correlation between those who used them and those who did not [F=3.418 (1, 102), p=.067, partial η2=.032]. Also, there was no significant association between IgM anti-nucleocapsid antibody levels in HCWs who did not use gloves and subjects who used them regularly or occasionally [F=0.123 (1, 102), p=.727, partial η2=.001].

Figure 2 the linear graphs present SARS-CoV-2 IgG antibodies in HCWs in the six subgroups during the three times assessed repeated every three months (three points of measuring). HCWs and non-medical workers who continuously used a surgical mask had lower values of IgG-anti-nucleocapsid antibodies in all measurements except for the first one (initial level). The assumption is that the initial level of IgG in the first three months represents the time of seroconversion to SARS-COV2. The values of IgG in the first and the second time points (time flow of about six months) are similar in subjects who used a respiratory mask and those who did not. However, at the third measurement, IgG-antibody values were higher in subjects who did not use a respiratory mask (10.63: 7.88; establishment of seroconversion). The titer of IgG- anti-nucleocapsid antibodies in subjects who did not use protective glasses was higher or approximately the same as those who used protective glasses regularly or sometimes. The values of IgG anti-nucleocapsid antibodies in the subjects who did not use the visor are higher- or approximately the same compared to the HCWs who used it regularly or occasionally. The values of IgG in HCWS who did not use a protective mantel are higher or approximately equal to those who used it. There was no statistically significant difference in IgG titer associated with putting on a mantel or not [F=1.574 (1, 102), p=0.212, partial η2=0.015]. Also, the values of IgG antibodies in HCWs who did not use protective gloves are higher or approximately equal to IgG in HCWs who used them. However, no statistically significant difference was found in IgG antibody values in subjects who did not use gloves and subjects who used them regularly and occasionally F=0.917 (1, 102), p=0.340, partial η2=0.009].

5. DISCUSSION

Significance of the study

In the first two months of the COVID-19 epidemic and pandemic, PPE was insufficient to prevent SARS-COV-2 transmission (12-15). So, healthcare workers become ill sooner or later infected (14-17). Hypothetically, PPE played an important role in preventing SARS-CoV-2 transmission among HCW before immune-prophylaxis by vaccination in BH, so the authors of this study wanted to evaluate the use of PPE in light of serological analyses of nucleocapsid antibodies IgM and IgG during three repeated measurements every three months.

Summary of findings

Increase SARS-COV-2 IgM titer three, six, or nine months after past COVID-19 infection and before vaccination. It presents a new acute or recent asymp-

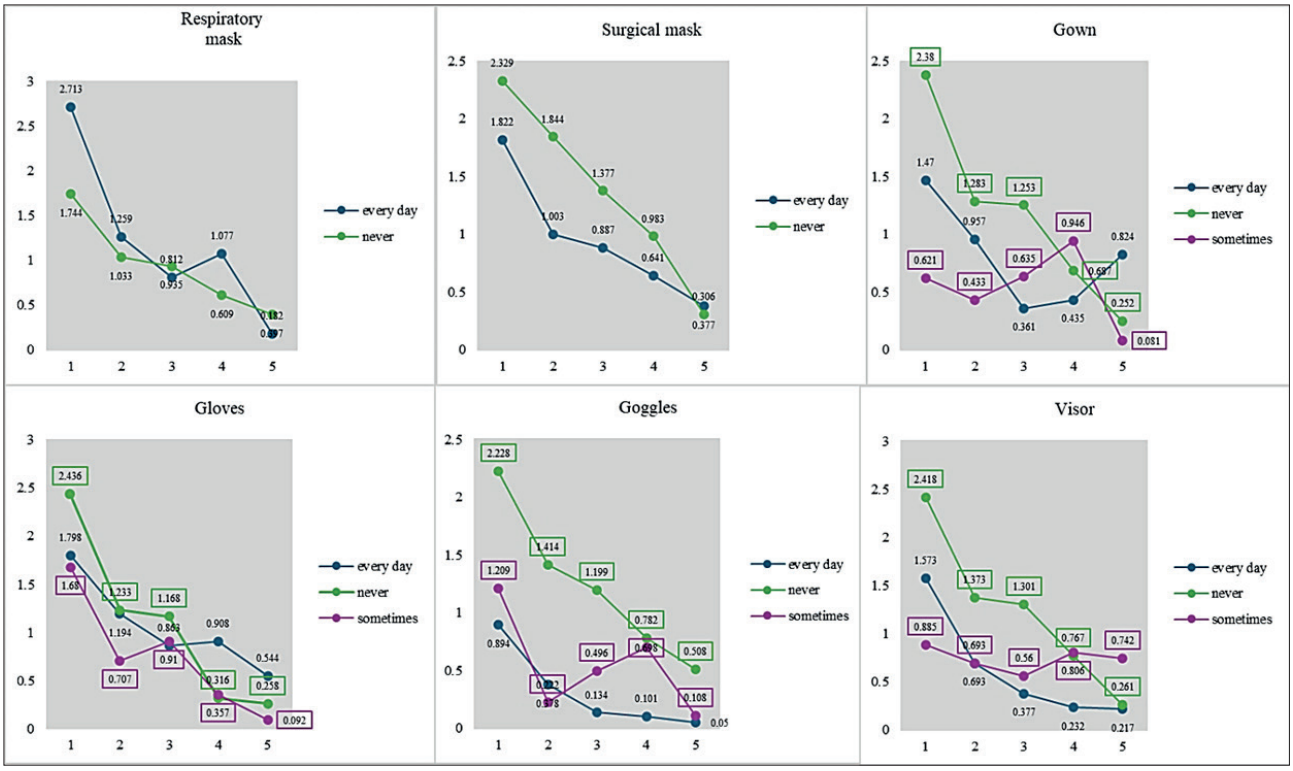

Figure 1. Profile of IgM antibody values based on the use of personal protective equipment (PPE) through five measurments. The X-axis shows the number of measurments at which the antibody value was determined. The Y-axis shows the value of antibodies in COI units. The frequency of using PPE is represented by the responses: every day, never and sometimes.

tomatic infection in our HCWs and unrecognized COVID-19. This implies considerable source and unrecognized risk of transmission of the SARS-COV-2 virus, and among HCWs, COVID-19 is an infectious disease with a high prevalence rate.

Explanation of findings

Consistent use of PPE has reduced SARS-CoV-2 transmission and delayed COVID-19 infection by serological findings (18-20). In 4927 health workers in pediatric hospitals in Türkiye who did not regularly use any mask or visor compared to those who used them found that seropositive were more common (21). Results among our HCWs who used protective equipment were generally lower than those who did not and agreed with the results of Oygar and collaborators (21). The prevalence of seropositive healthcare workers of 16.3% was found by Pinarlk et coauthors (22) following seroconversion among healthcare workers who used PPE. Similar results were found in our HCWs, who were infected with SARS-COV-2 at the beginning of the study. IgM increases in the first days of COVID-19 and presents an indicator for an acute or recent diagnosis of infection (23-24). That explains our seroprevalence results during three repeated measures. The IgM seroprevalence rate was high three months after confirmed COVID-19 infection and gradually decreased over the next 6 months (COI positive: 38.6% of IgM1, 17.4% of IgM2, and 13.4% of IgM3). This also means these HCWs had a new acute infection or reinfection of COVID-19 (25) as asymptomatic and unrecognized COVID-19. In this regard, asymptomatic individuals with seroconversion were from 13% to 39%, indicating a potentially high infection rate among healthcare workers (25) and their patients. Controversy IgM seroprevalence rate was higher among nonmedical workers than HCWs in all three measuring times (COI positive: 63% of IgM1, 42% of IgM2, and 32% of IgM3). The long-lived IgM- response may mean that the remaining virus continued to replicate, which was found among nonmedical workers. However, long-lived IgM- response at low levels stimulates the immune system to produce antibodies (26, 27), as confirmed in our HCWs. Beltrán and collaborators (28) found that 4.6%-5.2% of subjects who used PPE had a positive PCR test or IgM antibody to SARS CoV2.

A high seropositive nucleocapsid IgG antibody indicates previous exposure to the virus, after which the amount of IgG decreases (29, 28, 26, 27). Elevated titer of IgG at baseline is considered reinfection (27), which was confirmed in all our subjects (COI positive, HCWs vs. nonmedical workers vs. 93% of IgG1: 74% of IgG2: 81% of IgG3 vs. 91% of IgG1: 96% of IgG2: 92% of IgG3). Conversely, the IgG titer values are higher for our nonmedical workers than in HCWS. Pınarlk and collaborates (22) found a prevalence of seropositive healthcare workers of 16.3%. The degree of exposure to the SARS-CoV-2 virus is associated with higher seroprevalence regardless of the use of PPE (30). In a study that included police officers who only used a surgical mask, a detectable titer of IgG antibodies was established (31).

Strengths and limitations

This is the first study in the region that deals with the relationship between the preventive effects of using PPE, as shown by the seroprevalence of nucleocapsid SARS-COV-2 IgM and IgG antibodies in HCWs

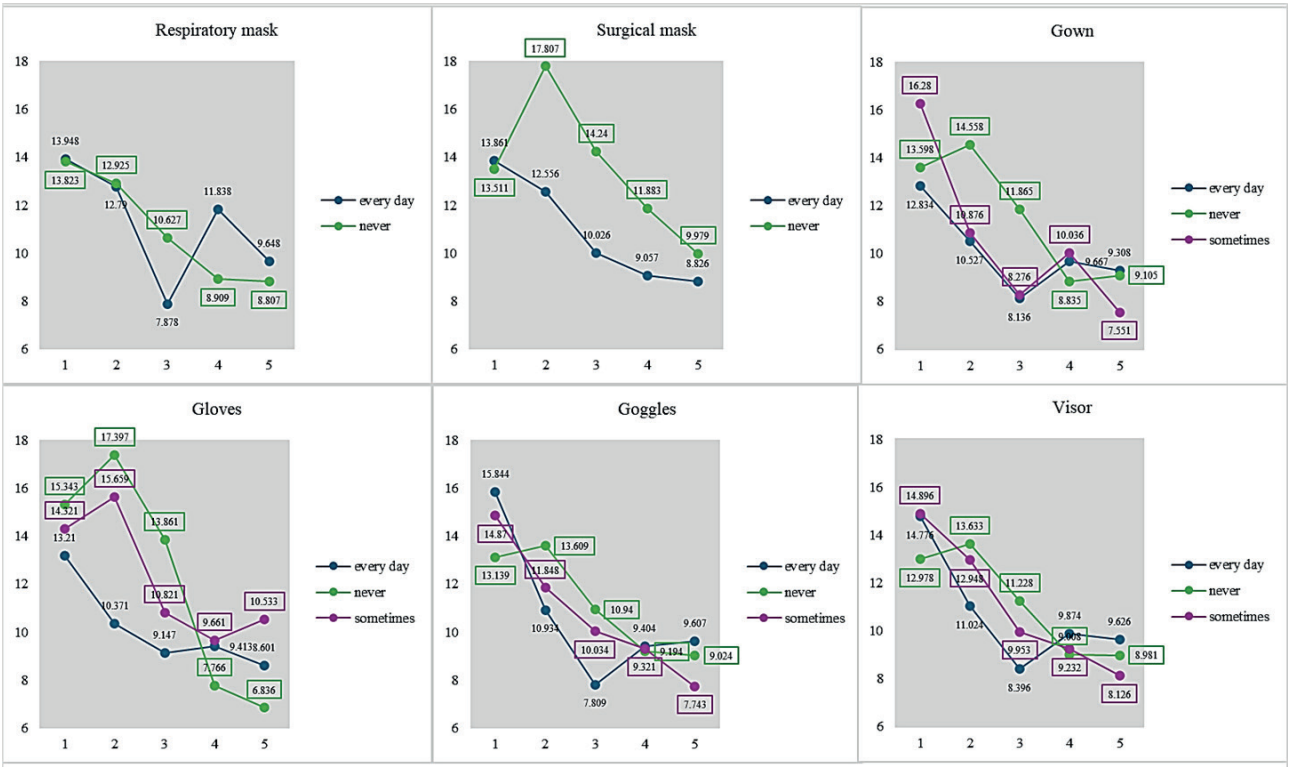

Figure 2. Profile of IgG antibody values based on the use of personal protective equipment (PPE) through five measurments. The X-axis shows the number of measurments at which the antibody value was determined. The Y-axis shows the value of antibodies in COI units. The frequency of using PPE is represented by the responses: every day, never and sometimes.

during twelve months and before vaccination. The longitudinal prospective design implies repeated measurements of IgM and IgG and analysis of cohorts and sub-cohorts. The weakness is the relatively small number of respondents who could not be followed to different departments and clinics of health institutions. It also remains questionable what is the serological response to the use of PPE in HCWs who previously had a severe clinical form of COVID-19.

Recommendations for clinical practice and future research

Every new emerging epidemic (or pandemic) represents a crisis beyond the control of the causative agent without vaccination and adequate treatment. Therefore, the PPE application requires every time and implementation of learned epidemiological preventive activities similar to the scenario during the recent COVID-19 pandemic. More future research is needed to evaluate the effectiveness of PPE, serological answers, relationship with other preventive activities, and factors that can influence this evaluation.

6. CONCLUSION

Healthcare workers who did not use PPE during the epidemic (or pandemic) of COVID-19 before vaccination had a higher titer of IgM anti-nucleocapsid antibodies, especially those who did not use protective glasses and visors. Although the titer of IgG anti-nucleocapsid antibodies was higher in subjects who did not use protective means, no significant difference was found in HCWs who used them.

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- **Conflict of interest:** None to declare.
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