

Original article

Seroprevalence of SARS-CoV-2 virus infection in employees in the health insurance sector

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Summary

Introduction. Serological testing for SARS-CoV-2 virus infection is a valuable method of estimating the extent of COVID-19 disease prevalence. The study aims to assess the seroprevalence of SARS-CoV-2 virus infection in a specific group of respondents employed in the health insurance sector, to determine the ratio of symptomatic and asymptomatic cases, as well as to examine susceptibility to COVID-19 in relation to the ABO blood group system.

Methods. This research was conducted among 150 randomly selected employees from the health insurance sector of the Republic of Srpska. All respondents completed the survey, voluntary consent to participate, and had a blood sample taken. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies.

Results. The seroprevalence of SARS-CoV-2 virus infection was 70.7%. Out of the 100 seropositive respondents, 48% had the presence of a symptom of COVID-19, while 52% had no symptoms of the disease in the period from 1 March 1 2020 until the day of testing. Examining the association between ABO blood group system and seropositivity in our study, we found that the highest seroprevalence of SARS-CoV-2 IgG antibodies among respondents was in blood group B (83.3%), followed by blood group AB (80.0%) and blood group A (75.0%), while the lowest seroprevalence was among respondents with blood group O (54.1%).

Conclusion. Among employees in the health insurance sector, SARS-CoV-2 seroprevalence of 70.7% was registered. Among respondents with a positive serological result on IgG, 52% were asymptomatic. The seroprevalence of SARS-CoV-2 virus infection is the lowest among subjects with blood group O.

Key words: COVID-19, seroprevalence, healthcare professionals, blood types

Introduction

Coronavirus disease 2019 (COVID-19), also known as novel coronavirus pneumonia (NCP), was first recorded in Wuhan, China in December 2019. At the beginning of March 2020, there were more than 80,000 infected people, of whom 3,200 died in China. According to the World Health Organization, from the beginning of the pandemic to 28 May 2021, 168,040,871 cases of COVID-19 and 3,494,758 deaths worldwide were confirmed. In Bosnia and Herzegovina, 203,658 cases and

9,184 deaths were confirmed in the same period [2]. In the Republic of Srpska, the first cases of COVID-19 were confirmed on 5 March 2020, and until 27 May 2021, 63,996 persons were tested positive, of whom 3,667 died [3].

COVID-19 infection rate and mortality rate vary between countries [4]. The exact reason for the different infection and mortality rates depending on the population is not clear. Several factors, such as age, gender, genetics, presence of comorbidities, and economic status, could be attributed to inequalities in susceptibility to COVID-19 disease and the severity of the clinical picture of the disease [4].

The beginning of epidemiological surveillance was accompanied by laboratory diagnostics. Over time, with the increase in the number of patients, both in the Republic of Srpska and around the world it was not possible to confirm all suspicious cases using RT-PCR test. In addition, as the epidemic progressed, asymptomatic cases of infection were found. According to Oran et al., asymptomatic infections account for approximately 40% to 45% of infections [5].

A study conducted by Korth and co-workers at the University Hospital of Essen between 25 March and 21 April 2020, found an overall IgG antibody prevalence among healthcare workers of 1.6%, being the highest in the group of healthcare workers with medium risk in relation to exposure [6].

A study conducted by Oliveira et al. investigated the prevalence of anti-SARS-CoV-2 antibodies in outpatients at a clinic in Sao Paulo, Brazil. This serological study included 439 patients from several outpatient services of which 61 patients were positive (13.9%). The prevalence of IgG was lower in patients who received a seasonal influenza vaccine [7].

In the midst of the 2019 SARS-CoV-2 pandemic, several brief scientific reports have been published examining the link between the ABO blood group system and the risk of SARS-CoV-2 virus infection [8]. Latz et al., as well as Zietz et al., found that individuals with blood group O were at the lowest risk for SARS-CoV-2 infection [9,10].

In December 2020, the Medical Faculties in Foca and Banja Luka and the Public Health Institute of the Republic of Srpska, with the approval of the Ministry of Health and Social Welfare of the Republic of Srpska, launched a national seroprevalence study of anti-SARS-CoV-2 antibodies. The study found that the seroprevalence in the Republic of Srpska was 40.3% [11].

So far, there have been no seroepidemiological investigations in specific groups in the Republic of Srpska, nor studies of the connection between the seroprevalence of SARS-CoV-2 and ABO blood groups. Therefore, this study aims to assess the seroprevalence of SARS-CoV-2 in a specific group of respondents employed in the health insurance sector, who do not work in the provision of health services, to determine the ratio of symptomatic and asymptomatic cases, and to examine the relationship between seroprevalence and ABO blood groups.

Methods

The study included 150 respondents (110 female and 40 male respondents) employed in the Health Insurance Sector of the Republic of Srpska, Banja Luka branch office, who do not work in the provision of health care services. After getting acquainted with the goals of the study, the respondents voluntarily accepted to participate in the study. The survey was conducted in March, 2021.

The collection of data and blood samples in the field was performed by trained health workers employed at the Faculty of Medicine in Foca. Laboratory analysis of samples was performed in the laboratory of the Faculty of Medicine Foca.

Participants answered questions from a two-part questionnaire. The first part of the questionnaire referred to the basic data on the respondent. In the second part of the questionnaire, respondents answered questions related to COVID-19, symptom history, hospitalization, testing and PCR and/or rapid antigen or serological test results, vaccination, and the presence of symptoms in household members.

A blood sample was taken from all respondents by peripheral venipuncture. Blood samples were taken from 8 to 11 a.m. After venipuncture, the blood was left for 30-35 minutes at room temperature to coagulate spontaneously. The serum was separated after centrifugation at 3000 rpm for 10 min, the serum was separated. The sera were transported in the cold chain regime to the Faculty of Medicine in Foca on the same day.

Serum samples were tested for the presence of specific antibodies to SARS-CoV-2 IgG using ELISA method by commercial tests EUROIM-MUN Medizinische Labordiagnostika AG, Germany. The measurement was performed on a EUROIMMUN ELISA Analyzer I-2P ("Euroimmun AG"). EUROIMMUN ELISA SARS-CoV-2 At is an ELISA test for the semi-quantitative detection of IgG antibodies to SARS-CoV-2 virus in human serum or plasma. The cut-off value recommended by the manufacturer for positivity is >1.1. The test has a sensitivity of 94.36% and a specificity of 100%.

The seroprevalence of SARS-CoV-2 virus infection was assessed as the proportion of individuals who had positive results to a serological test for the presence of specific IgG antibodies. Descriptive statistical measures are presented for the basic characteristics of the respondents. Pearson's chi-square test was used to determine the difference between categorical variables, while the parametric Student's t-test was used to determine the difference between quantitative variables. Pearson's chi-square test was used to determine the difference in the frequency of seroprevalence between blood groups. Student's t-test was used to determine the difference in antibody levels of seropositive respondents according to age and in relation to the presence

and/or absence of symptoms. All tests refer to two-way testing. The cut-off value for determining the existence of a statistically significant difference is p<0.05.

Results

The study involved 150 respondents, of whom 40 (26.7%) were male and 110 (73.3%) were female. The educational structure showed that 122 respondents (83%) were highly educated, while 25 (17%) respondents had secondary education. Respondents were divided into two categories according to age. In the age group up to 40, there were 74 respondents (49.7%), while in the group of respondents older than 40 there were 75 of respondents (50.3%). The youngest respondent was 21 and the oldest 64 years old. The seroprevalence of SARS-CoV-2 virus infection in healthcare employees was 70.7%. The seroprevalence in men was higher and amounted to 77.5%, and in women it was 68.2%.

Regarding the mean values of the levels of specific IgG antibodies to SARS-CoV-2, no statistically significant difference was found between the category older than 40 (4.55) compared to the category younger than 40 (3.55), (t=1.811, DF=98, p=0.073). Out of 100 seropositive subjects, 48% of them had the presence of some symptoms of COVID-19, while 52% of them did not have any symptoms of the mentioned disease in the period from 1 March 2020 until the day of testing. The mean value of the titer of specific antibodies to SARS-CoV-2 IgG in respondents with the presence of symptoms was 3.37, while the mean value of the titer of specific antibodies to SARS-CoV-2 IgG in respondents without the presence of symptoms was 4.68 (Figure 1). The analysis of these data indicates that the mean values of the titer of specific antibodies to SARS-CoV-2 IgG of seropositive respondents were statistically significantly higher in those who did not have symptoms of COVID-19, compared with respondents with symptoms of COVID-19 (t=2.724,

DF=96, p=0.008). Out of the total number of respondents, 130 of them mentioned their blood group. The results of the study of the distribution of seropositive and seronegative subjects and seroprevalence by blood groups are shown in Table 1. From the results shown in Table 1, it can be seen that the highest frequency of seropositive respondents was in respondents with blood group A, while the largest number of respondents with seronegative results belonged to blood group O.

Out of the total number of respondents, 46 (33.6%) were tested by RT-PCR test, while 54 (41.5%) were tested by rapid antigen or sero-logical test. These tests results are shown in Table 2.

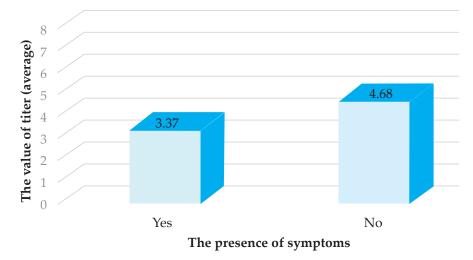


Figure 1. Mean values of the titer of specific antibodies to SARS-CoV-2 IgG in seropositive respondents in relation to the symptoms of COVID-19

Pland group	The result of serological testing		Chi-	n wiluo	IgG seroprevalence	
Blood group	Positive	Negative	square	p-value	to SARS-CoV-2	
А	36 (40.0%)	12 (30.0%)	8,649	0.034	75.0%	
В	20 (22.2%)	4 (10.0%)			83.3%	
AB	8 (8.9%)	2 (5.0%)			80.0%	
О	26 (28.9%)	22 (55.0%)			54.1%	
Total	90 (100.0%)	40 (100.0%)			69.2%	

Table 1. Ratio between blood groups and serological testing results, IgG seroprevalence for SARS-CoV-2

Table 2. Results of previous testing by RT PCR test and/or rapid antigen or serological test

True of guariana testing	Number of tested out of the total	Previous test result N (%)	
Type of previous testing	number of respondents N (%)	Positive	Negative
COVID-19 RT-PCR test	46 (33.6)	23 (16.3)	23 (16.3)
Rapid antigen or serological test	51 (40.2)	26 (18.4)	25 (17.7)

Out of the total number of respondents, 46 (33.6%) of them stated that they were tested by PCR test in the period from 1 March 2020 until the day of testing for SARS-CoV-2 IgG. Com-

paring the frequency of seropositivity between the group of PCR positive and PCR negative subjects, a statistically significant difference was found (x2 = 4.487, DF = 1, p = 0.028) (Table 3).

Table 3. Results of RT PCR testing in relation to the qualitative value of serological testing for SARS-CoV-2 IgG

			Qualitative value of IgG (positive or negative)		Total
			Positive	Negative	1000
Was the PCR test positive?	Yes	Ν	19	4	23
		%	61.3	26.7	50.0
		% out of Total	41.3	8.7	50.0
	No	Ν	12	11	23
		%	38.7	73.3	50.0
		% out of Total	26.1	23.9	50.0
		Ν	31	15	46
Total		%	100.0	100.0	100.0
		% out of Total	67.4	32.6	100.0

Out of the total number of respondents in our study, 9 (6.4%) have received the first dose of a vaccine, 3 respondents (33.3%) have received the second dose of a vaccine. Among our respondents, 64 (46.7%) stated that some members of their family had some of the symptoms of COVID-19 from 1 March 2020 until the day of testing, out of which 39 (60.9%) were proven by RT-PCR test (Table 4).

Table 4. Presence of symptoms in respondents and their family members and vaccine coverage

	Yes N (%)	No N (%)	Total	
Have you had any symptoms of COVID-19 from 1 March 2020 until the day of testing?	69 (46.3)	80 (53.7)	149	
Have any of your family members had any of the symptoms of COVID-19 since 1 March 2020?	64 (46.7)	73 (53.3)	137	
If yes, has their infection been confirmed by PCR?	39 (60.9)	25 (39.1)	64	
Have you received the first COVID-19 vaccine dose?	9 (6.4)	132 (93.6)	141	
	Sputnik V	Pfizer-BioNTech		
If yes, which vaccine have you received?	7 (77.8)	2 (22.2)	9	
Have you received the second vaccine dose?	3 (33.3)	6 (66.7)	9	

Discussion

Our study was conducted in late March 2021, in order to assess the seroprevalence of SARS-CoV-2 virus infection in a specific group of respondents employed in the health insurance sector, who by the nature of their work do not work in the provision of healthcare services, and to determine the ratio of symptomatic and asymptomatic cases, and to examine susceptibility to COVID-19 in relation to the ABO blood group system.

A total number of 150 randomly selected respondents participated in the study, of which 40 (26.7%) were male and 110 (73.3%) were female. The seroprevalence of SARS-CoV-2 virus infection in the study group was 70.7%.

Having in mind the importance of assessing the seroprevalence of SARS-CoV-2 virus infection for the purposes of public health activities and planning, a number of studies on this topic have been conducted worldwide. Thus, in the period from 25 March to 21 April, 2020, Korth et al. conducted a study in Germany among 316 health professionals who have direct contact with COVID-19 patients. In the study population, the seroprevalence was 1.6%, as evidenced by a semi-quantitative ELISA test [6].

Iversen et al. conducted a seoprevalence study among specific categories of the population, such as voluntary blood donors and health-care workers. The study was conducted in Denmark in the period from 15 April to 23 April, 2020. The seroprevalence among healthcare workers was higher than that of voluntary blood donors and amounted to 3.04% [12].

In a study conducted between 5 May and 15 May 2020 in Northern Italy among 6,075 subjects, the seroprevalence of SARS-CoV-2-IgG was 23.1% [11]. In April 2020, a screening of employees (1,500 respondents) was performed in 22 nursing homes in Stockholm with a rapid COVID-19 test to detect specific antibodies to SARS-CoV IgM and IgG. Seropositive employees were found in 21 out of the 22 nursing homes. The seroprevalence of SARS-CoV-2 IgG antibodies in the study population was 23%, while 14.3% of the respondents were positive for IgM antibodies (alone or in combination with IgG), indicating the recent presence of infection. Of those who were seropositive, 46.5% reported no clinical symptoms indicating asymptomatic infection [14].

Grant et al. conducted a seroprevalence study in healthcare workers in London in the period May-June 2020, the seroprevalence of SARS-CoV-2-IgG was 31.6% [15].

A systematic literature review conducted by Rostami et al. included 47 studies, in which 399,265 people from 23 countries participated. Heterogeneity was observed among the studies (I2=99.4%, p<0.001). The seroprevalence of SARS-CoV-2 in the general population varied from 0.37% to 22.1%, with a cumulative estimate of 3.38% (95% CI 3.05-3.72%; 15 879/399 265) [16].

The seroprevalence of SARS-CoV-2 infection registered in our study has higher values than those published so far, both among the groups exposed to patients and among the unexposed. Our respondents do not work on providing health care, but in the front offices they have the possibility of contact with patients who come to the institution for various certificates in order to exercise their rights in the field of healthcare protection. The higher values of SARS-CoV-2 infection seroprevalence can be explained by the fact that our research was conducted during the third wave of the pandemic, while the other mentioned studies were conducted during the first or the second wave, almost a year earlier than our research. In addition, at the time of conducting the cited studies, immunization against COVID-19 had not yet been initiated, which in our case could have had an effect on increasing seroprevalence. Out of the total number of seropositive respondents in our study, 52% of them did not report any symptoms of COVID-19, which is in accordance with a study conducted by Johanna et al. in Stockholm during April 2020 [14].

Previous reports have shown an association of ABO blood group systems with susceptibility to a wide range of infections such as severe acute respiratory syndrome infection with SARS-CoV-2 virus, West Nile virus, Human Immunodeficiency virus, Hepatitis B and Norwalk virus [14]. The possible association between the ABO blood group system and COVID-19 infection and mortality was highlighted. Blood group A has been shown to be a risk factor for the development of COVID-19 disease, while blood group O has a lower incidence rate than the mentioned disease [17]. Similar results were obtained in a study by Latz et al. [9], as well as Zietz et al. [10]. The study conducted by Latz et al. found the results showing that blood group A did not correlate with positive COVID-19 test results (AOR:1.00, CI:0.88-1.13), while blood groups B and AB were associated with a higher probability of a positive COVID-19 test result (AOR:1.28, CI:1.08-1.52; AOR:1.37, CI:1.02-1.83). Blood type O is associated with a lower probability of a positive COVID - 19 test result (AOR: 0.84, CI: 0.75-0.95) [9].

The study conducted by Zietz et al. showed a slightly increased prevalence of Coronavirus infection in people who were not blood group O. The risk of intubation was lower in people with blood group A, and increased in people with blood groups AB and B compared to people with blood group O. A higher probability of mortality was found in people with blood group AB, and lower in people with blood groups A and B [10].

Several pathophysiological mechanisms of association between the ABO blood group system and SARS-CoV-2 virus infection have been proposed [18]. Anti-A and/or anti-B antibodies, which are, for example, present in individuals with O group, can bind to A and/or B antigens expressed on the viral envelope, thus preventing infection of target cells, or these naturally occurring antibodies could function as antibodies to neutralize the virus [18]. If these assumptions are correct, differences in initial susceptibility to SARS-CoV-2 infections could be explained in this way. Zhao et al. found that the incidence of COVID-19 infection was higher in people with AB blood group [19], but Zietz and Tatonetti found that blood group AB was associated with reduced COVID-19 infection [10]. Moreover, during 2020, Zhao et al. found that blood group A was associated with higher mortality of COVID-19, and blood group O with lower mortality [19].

Examining the association between ABO blood group system and seropositivity in our study, we found that the highest seroprevalence of SARS-CoV-2 virus infection was among respondents with blood group B and it was 83.3%, while lower blood values were recorded in other blood groups. Thus, the seroprevalence in respondents with blood group AB was 80%, while respondents with blood group A had a seroprevalence of 75%. Respondents with O blood group had the lowest seroprevalence (54.1%), which is in accordance with research in France and Turkey. Gallian et al. in a study conducted in France showed that people with blood group O have a lower seroprevalence rate of SARS-CoV-2 virus infection [20], which was also shown by a study conducted by Göker et al. in Turkey in April 2020. [21]. Further research is needed to explain the reasons for the protective role of blood group O.

Zeng et al. in their study did not find any association between ABO blood types and mortality from COVID-19. The contradiction of the obtained results could be due to the small size of the sample, the residual conclusion from the heterogeneity of the population, the difference in the region, etc. [22].

While the diagnosis of acute SARS-CoV-2 infection is performed by RT-PCR test in respiratory samples, there is a growing demand for serological tests for population-based epidemiological studies and for the assessment of infection in individuals [23]. Recent studies have confirmed the suitability of various commercial immunotests including a high-throughput platform with a random approach for determining SARS-Cov-2-IgG in patients with COVID-19 [23]. Determination of IgG antibodies to SARS-Cov-2 is the method of choice for assessing the seroprevalence of SARS-CoV-2 virus infection. Measurement of SARS-CoV-2-IgG using automated immunoassays allows rapid testing of a large number of samples [24].

Study limitations

The study has certain limitations, because it did not include the examination of cellular immunity. In addition, memory bias in responding to the presence of COVID-19 symptoms is possible. Also, other respiratory infections may have been present during the observed period, so the reported symptoms may be attributed to the other infections. However, the study certainly provided important findings and contributed to a better understanding in this field.

Conclusion

The research showed that the frequency of seroprevalence of SARS-CoV-2 virus infection among the population of employees in the healthcare sector is 70.7%. Out of the total number of seropositive subjects, 52% were asymptomatic, and seropositivity was the lowest in people with O blood group.

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Ethical approval. The Ethics Committee of the Faculty of Medicine Foca approved the study and informed consent

was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Seroprevalencija infekcije virusom SARS-CoV-2 kod zaposlenih u sektoru zdravstvenog osiguranja

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Uvod. Serološka ispitivanja infekcije virusom SARS-CoV-2 su dragocjena metoda procjene obima raširenosti COVID-19 oboljenja. Studija ima za cilj da procijeni seroprevalenciju infekcije virusom SARS-CoV-2 u specifičnoj grupi ispitanika zaposlenih u sektoru zdravstvenog osiguranja, da odredi omjer simptomatskih i asimptomatskih slučajeva, kao i da ispita osjetljivost na COVID-19 u odnosu na AB0 sistem krvnih grupa.

Metode. Ovo istraživanje je sprovedeno među 150 slučajno odabranih zaposlenih radnika iz sektora zdravstvenog osiguranja Republike Srpske. Svi ispitanici su popunili anketu, dali dobrovoljni pristanak za učešće i uzet im je uzorak krvi. Uzorci seruma su testirani na prisustvo specifičnih IgG antitijela na SARS-CoV-2.

Rezultati. Seroprevalencija infekcije virusom SARS-CoV-2 je bila 70,7%. Od 100 seropozitivnih ispitanika, njih 48% je imalo prisustvo bilo kog simptoma COVID-19, dok 52% nije imalo nijedan simptom navedenog oboljenja u periodu od 1. marta 2020. godine do dana testiranja. Ispitivanjem povezanosti između AB0 sistema krvnih grupa i seropozitivnosti u našem ispitivanju došli smo do rezultata da je najveća seroprevalencija SARS-CoV-2 IgG antitijela među ispitanicima bila kod krvne grupe B (83,3%), zatim kod krvne grupe AB (80,0%) i krvne grupe A (75,0%), dok je najmanja bila među ispitanicima 0 krvne grupe (54,1%).

Zaključak. Kod zaposlenih iz sektora zdravstvenog osiguranja registrovana je seroprevalencija infekcije virusom SARS-CoV-2 od 70,7%. Među ispitanicima sa pozitivnim serološkim rezultatom na IgG 52% je bilo asimptomatsko. Seroprevalencija infekcije virusom SARS-CoV-2 je najmanja među ispitanicima sa 0 krvnom grupom.

Ključne riječi: COVID-19, seroprevalencija, zdravstveni radnici, krvne grupe