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CLINICAL STUDY

COVID-19 symptom duration predicts immunoglobulin G seropositivity

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ABSTRACT

OBJECTIVES: The study focused on the relationship between routine clinical characteristics and anti-SARS-CoV-2 specific antibodies in a pilot sample of healthcare workers (HCWs) having suffered COVID-19. The aim was to investigate the existence of readily available predictors of antibodies against COVID-19.

METHODS: As part of the recognition of COVID-19 as an occupational disease in 152 HCWs with the mean age of 43.2 years, personal, anthropometric and anamnestic data related to the disease as well as anti-spike immunoglobulin (Ig) levels were obtained. Through descriptive statistics, correlation and regression analyses, relationships of all variables and Ig levels, especially seropositivity of IgG, were investigated.

RESULTS: The mean interval between the symptom onset and the determination of antibodies was 58 days. IgG seropositivity and IgM seropositivity were noted in 82 % and 49 % of HCWs, respectively. Symptom duration was the only statistically significant predictor of IgG seropositivity. With each day of symptom duration, the probability of IgG seropositivity increased from 1.078 to 1.092 times ($p < 0.05$). If symptoms lasted longer than 17 days, a majority (almost 80 %) of the subjects demonstrated seropositivity in the following months.

CONCLUSION: The presence of IgG immunity may be assumed from symptom duration. Such easy recognizing of seropositive patients may be a useful tool, e.g. in vaccination strategies *(Tab. 3, Fig. 1, Ref. 28)*. Text in PDF *www.elis.sk*

KEY WORDS**:** SARS-CoV-2, COVID-19, seropositivity, antibody, immunoglobulin, predictor.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has already contributed to millions of deaths worldwide (1). To expand the possibilities of prevention and treatment of this disease, it is essential to deepen the knowledge about the immune mechanisms applied in a COVID-19-infected organism. In most infected patients, the protective immune response, which is represented by both the production of neutralizing antibodies against surface and internal proteins of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and by cell-mediated responses, is induced following the infection. Both the humoral and cellular responses play a substantial role in the protection against the disease and are essential for SARS-CoV-2 clearance (2–4). Available evidence, yet sometimes conflicting, suggests that these responses generally persist several months after the acute phase of COVID-19 (3, 4). Various factors including disease severity may contribute to the magnitude of antibody response and affect the cell-mediated responses (3, 4).

Although immunity largely depends on the presence of specific antibodies (mostly immunoglobulin (Ig) G from a long-term point of view), their absence does not necessarily mean the absence of COVID-19 immunity. Memory B cells could provide durable humoral immunity even if serum neutralizing antibody titers decline (5, 6). T cells also play a crucial role, especially to overcome the acute phase. Specific T-cell response against SARS-CoV-2 is important for the recognition and killing of infected cells, particularly in the lungs of infected individuals (7). SARS-CoV-2-specific T-cell responses in patients, who recovered from COVID-19 suggest the potential for a durable T-cell-mediated immunity (8). However, virus neutralizing antibodies are likely to be a key metric for protection against infection by viruses such as SARS-CoV-2 and, therefore, data on predictors and kinetics of virus neutralizing antibody responses are needed (4, 9).

Healthcare workers (HCWs) are at high risk of contracting the infection and disseminating it further among both patients and colleagues (10). The prevalence of COVID-19 among HCWs significantly exceeds the prevalence of this disease in the general population, with front-line HCWs being the most commonly infected personnel (10). The recognition of COVID-19 as an occupational disease (OD) and its compensation depends on the legislation of

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each country. In the Czech Republic, clinically manifested and laboratory-confirmed infectious diseases, including COVID-19, may be recognized as ODs in employees for whom a hygienic-epidemiological investigation shows an increased risk of acquiring the disease while practicing the profession compared to the general population (11, 12).

The present work focused on the relationship between routinely determined personal, anthropometric and anamnestic characteristics related to suffered COVID-19 and anti-SARS-CoV-2 specific antibodies, mainly immunoglobulin (IgG) G, in the period after the disease in a pilot sample of HCWs. The aim was to investigate the existence of readily available predictors of antibodies against COVID-19 with respect to their clinical use.

Materials and methods

Study population

The pilot sample of the cross-sectional study consisted of 152 HCWs from the Olomouc Region, who were examined after the COVID-19 disease in order to recognize their disease as an OD at the OD Center of the Department of Occupational Medicine, Olomouc University Hospital between April and December 2020. The studied HCWs (127 females, 25 males, with the mean age of 43.2 (median 45) years) brought a report about the course of their disease from a general practitioner and were examined at the OD Center according to a uniform schedule, including the collection of antibodies against SARS-CoV-2. In the HCWs, viral RNA was detected from a nasopharyngeal swab through a reverse transcription polymerase chain reaction (PCR) test in the acute phase of the disease. All included cases were symptomatic. None of the HCWs were vaccinated against COVID-19 at the time of the examination. The HCWs were asked about all the symptoms stated by the World Health Organization (WHO) and their duration (13). Symptom duration was calculated by subtracting the symptom recovery date and the symptom onset date. Information provided by the patient was validated against the report from a general practitioner. Disease severity was also assessed according to the WHO classification (13).

All subjects signed an informed consent form regarding the anonymous use of their data. The study was approved by the Ethics Committee of the University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacky University Olomouc (reference no. 18/21).

Laboratory analysis

Neutralizing antibodies were determined using automated SARS-CoV-2 chemiluminescent assay by Diasorin – LIAISON XL kit (Saluggia, Italy). LIAISON XL detects serum anti-SARS-CoV-2 spike glycoprotein (S1 and S2) antibodies. For the diagnostic assay, the manufacturer states clinical sensitivity: 98.7 %, clinical specificity: 99.5 %; correlation with microneutralization test: positive-percent agreement: 100 %, negative-percent agreement: 96.9 % (14). Independent studies proved a comparable performance (both sensitivity and specificity) of Diasorin LIAISON XL with other commercial immunoassays (15, 16). The level of IgG antibodies was considered negative at < 15 AU/mL, positive at ≥ 15 AU/mL. For IgM antibodies, an index < 1.1 represented seronegativity, an index ≥ 1.1 represented seropositivity. Antibody detection and PCR testing were performed in an accredited microbiological laboratory of the university hospital in compliance with all standard procedures and manufacturers' instructions of used diagnostic sets and devices.

Statistical analysis

Statistical analyses were conducted in the R software environment (R Foundation for Statistical Computing, Austria; http://www.r-project.org/). All numerical variables were characterized with descriptive statistics. Studied variables, especially antibody levels, showed a right-skewed distribution as evidenced by the mean-median index >> 1 (Tab. 1). Correlations of selected variables with antibody levels were quantified with Spearman's correlation coefficient (r) and the level of significance (p) was determined. The dependence of IgG levels on personal, anthropometric and anamnestic data was explored by regression analysis methods. Seropositivity (or seronegativity, inversely, as a disjunct event) was a response variable in all regression models. Specifically, binomial logistic regression was used to identify predictors of seropositivity, in which other examined variables served as explanatory variables (potential predictors) of seropositivity. Another regression model was a proportional hazard regression (time-to-event analysis with interval censoring, Cox's regression), where the response variable consisted of two components – seronegativity (a decrease of antibodies below a cut-off) and the time interval from symptom onset until the serology test. In other words, this model predicted the level of IgG bound to a specific interval from the symptom onset. In this regression model, explanatory variables also included all remaining variables. Through classification and regression trees (CART) with variable settings as in Cox's regression, a cut-off for symptom duration statistically significant for the persistence of seropositivity was explored. The method investigates all the possible cut-offs within ranges of each explanatory variable to maximize the difference between the posterior distribution of occurred seronegativity. Kaplan-Meier curves (Fig. 1) describe the posterior distribution of seronegativity and the criterion of difference maximization is determined by log-rank statistics.

Tab. 1. Studied characteristics of subjects.

(n)	152 (127 females, 25 males)	
Characteristics	Mean (95 % CI)	Median
Age (years)	43.21 (41.60; 44.82)	45
Weight (kg)	77.74 (74.72; 80.76)	73.5
Height (cm)	169.95 (168.63; 171.27)	169.5
BMI (kg/m^2)	26.8 (25.88; 27.73)	25.1
IgG_value (AU/mL)	53.78 (47.23; 60.32)	42.25
IgM_value (index)	4.12 (2.33; 5.9)	0.93
Symptom duration (days)	17.67 (15.57; 19.77)	14
Interval between SARS-CoV-2 detection and serology test (days)	56.34 (51.55; 61.13)	52
Interval between symptom onset and serology test (days)	57.99 (53.24; 62.75)	54

CI – confidence interval; BMI – body mass index; IgG – immunoglobulin G; IgM – immunoglobulin M; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2

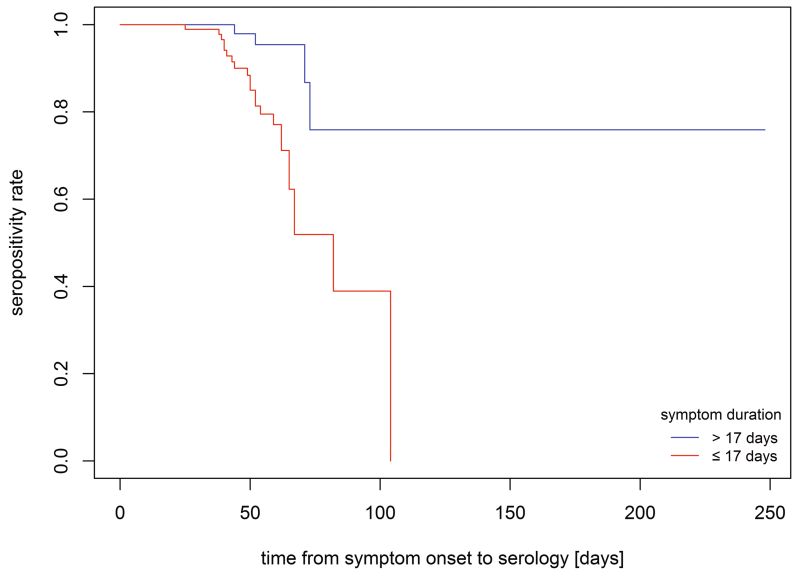

Fig. 1. Depiction of CART with Kaplan – Meier curves as the maximum difference in the curves (according to the log-rank test) showing the seropositivity rate depending on the time interval from symptom onset to serology testing. CART – classification and regression trees.

Results

Characteristics of the study population

The basic characteristics of all the subjects are shown in Table 1. According to body mass index (BMI; average 26.8, median 25.1 kg/m^2), the studied HCWs were slightly overweight. The interval between diagnostic swab sampling and serology testing in the study group averaged 56.3 (median 52) days, while the average interval between symptom onset and serology testing was 58 (median 54) days. In other words, HCWs in the study were diagnosed with COVID-19 usually on day 2 after the onset of symptoms. The (acute) symptoms of the disease lasted on average 17.7 (median 14) days. Most of the studied population (72.4 %) developed a mild course of COVID-19. A moderate course of the disease was recorded 42 times (27.6 % of HCWs). A severe or critical course of the disease did not occur in any HCW. Symptom duration did not differ significantly between mild and moderate forms.

The mean value of IgG antibodies in the whole sample was 53.8 (median 42.3) AU/mL (i.e. above the cut-off for seropositivity), however 27 (17.8 %) of the subjects showed seronegativity at the time of serology testing. The mean value of the IgM index was 4.1 (i.e. above the cut-off for seropositivity), while the median IgM index was 0.93, which indicates seronegativity. Thus, low levels of IgM antibodies were detected in more than half of the sample, specifically in 78 subjects. The only statistically significant correlations found occurred between IgG values and age ($r = 0.23$, $p < 0.01$), symptom duration ($r = 0.2$, $p = 0.01$), weight ($r = 0.18$, $p = 0.03$), BMI ($r = 0.17$, $p = 0.04$) and the number of symptoms present ($r = 0.16$, $p = 0.049$). In the case of IgM, the correlations of all the investigated numerical variables were of no statistical significance.

Predictors of seropositivity

From binomial regression, only one statistically significant predictor of IgG seropositivity emerged, namely the duration of disease symptoms (Tab. 2). With each day of symptom duration, the probability of IgG seropositivity increases 1.078 times, $p = 0.017$. The effect of the time interval from symptom onset to blood sampling for serology analysis on seropositivity was not statistically significant in the study sample. However, considering this time interval along with IgG levels in Cox's regression model revealed only one statistically significant predictor for maintaining protective IgG levels, again symptom duration (Tab. 3). According to this model, every day of symptom duration increased the probability of IgG levels persisting above the seropositivity cut-off 1.092 times, $p = 0.002$. In other words, the chance of IgG seronegativity decreased 0.916 times per each day of symptom manifestation.

A detailed analysis of the only significant predictor of IgG seropositivity using the CART approach employing Cox's regression showed that the biggest difference in the seropositivity rate occurred over a symptom

Tab. 2. Binomial logistic regression of seropositivity prediction. Symptom duration was the only statistically significant predictor.

Explanatory variable/predictor	OR	95 % CI	SE	z-value	p-value
Age	0.983	0.934–1.033	0.025	–0.678	0.498
Sex = female	0.763	0.214–2.396	0.606	–0.447	0.655
BMI	1.023	0.942–1.125	0.045	0.509	0.611
Number of symptoms present	1.057	0.844–1.326	0.114	0.488	0.626
Disease course	2.492	0.631–12.749	0.747	1.223	0.221
Symptom duration	1.078	1.021–1.155	0.032	2.381	0.017
Interval between symptom onset and serology test	1.001	0.985–1.026	0.010	0.113	0.91

OR – odds ratio; CI – confidence interval; SE – standard error; BMI – body mass index

Tab. 3. Proportional hazard (Cox´s) regression expressing the chance of seronegativity (or seropositivity as an inverse value) with respect to the interval from symptom onset to the serology test. Symptom duration was the only statistically significant predictor.

Explanatory variable/predictor	OR	95 % CI	SE	z-value	p-value
Age	1.024	0.979–1.07	0.023	1.029	0.303
Sex = female	1.405	0.436–4.531	0.597	0.569	0.569
BMI	0.958	0.876–1.048	0.046	–0.928	0.354
Number of symptoms present	1.082	0.904–1.295	0.092	0.858	0.391
Disease course	0.491	0.122–1.982	0.712	–0.999	0.318
Symptom duration	0.916	0.867–0.967	0.028	–3.159	0.002

OR – odds ratio; CI – confidence interval; SE – standard error; BMI – body mass index

duration of 17 days (log-rank test, p = 0.003) (Fig. 1). Subjects with symptom duration of more than 17 days had a seropositivity rate exceeding 90 % after 60 days from symptom onset. Almost 80 % of them with no sign of a decrease below the cut-off in case of a longer recorded interval from symptom onset to serology testing. In contrast, in those with symptoms lasting for a maximum of 17 days inclusive, approximately 60 days after symptom onset, IgG antibodies were present in protective concentrations in only half of the subjects with a significant decrease in the seropositivity rate in case of a longer recorded interval between symptom onset and serology testing (Fig. 1).

Discussion

The obtained results showed that the only statistically significant predictor of anti-SARS-CoV-2 IgG seropositivity in our study sample was symptom duration. In particular, symptom duration exceeding 17 days proved to be most important for maintaining a sufficient level of IgG antibodies. With each day of the acute phase of the disease, the probability of IgG seropositivity increased from 1.078 to 1.092 times, depending on the statistical model used. These results were obtained in a population of HCWs, whose antibody analysis was performed on average 58 days after the onset of COVID-19 symptoms.

As indicated in the Introduction section, data on immune responses after undergoing COVID-19 are often conflicting. A number of available studies, which associated antibody immunity after COVID-19 with disease severity, usually reported an earlier antibody response to more severe disease, specifically for IgG; a shorter time to peak IgG titers with higher values and that IgG persisted for longer in a severe disease compared to milder cases (4). However, in our study, disease severity was not proved to be a statistically significant predictor of persistent seropositivity. Comparisons between studies in this regard are greatly complicated by the inconsistent definition of disease severity. In the present work, the WHO classification was applied, which does not consider the duration of the acute phase of the disease (13).

It is the relationship between the duration of the acute phase of the disease, which was a significant predictor of seropositivity in the present work, and the antibody response after SARS-CoV-2 infection that has been addressed in relatively fewer studies. In the study by Wu et al conducted at the hospital discharge of 175 COVID-19 convalescents, the mean symptom duration noted in the highest quartile of IgG levels was 3 days longer than the duration in the lowest quartile (17). Chen et al charted longitudinal antibody responses to SARS-CoV-2 in 92 subjects after symptomatic COVID-19. Antibody responses to SARS-CoV-2 were unimodally distributed over a broad range, with symptom duration very weakly and insignificantly correlating with virus-specific IgG (r = 0.15) (18). The value of Spearman's coefficient was similar to our result (r = 0.2). A similar positive correlation between the symptom duration and anti-SARS-CoV-2 IgG (r = 0.16, p = 0.018) was noted by Bošnjak et al in their study of 50 convalescent individuals sampling 33 days post symptom onset (19). However, a different statistical method than the correlation analysis was not used to explore the relationship between symptom duration and seropositivity by both Chen et al and Bošnjak et al (18, 19). In our study, the correlation coefficient compared to the other cited was therefore the highest one, although symptom duration and IgG values seemed to correlate only poorly, but with statistical significance. These results and publicly available data suggest that a certain threshold of disease duration might be required for the successful mounting of neutralizing anti-SARS-CoV-2 humoral responses (19). No study was found in available literature that looked in more detail at the duration of COVID-19 symptoms, especially at the determination of its potential cut-off, and its relationship to the persistence of seropositivity, as the present work has done.

IgG dynamics appear to follow a pattern of peak (within the first month from symptom onset), plateau, and persistence at lower levels beginning approximately from 7 weeks after symptom onset up to months; however, various studies differ in the particular intervals (4, 20–22). In our study, the mean interval from symptom onset to IgG analysis was approximately 8 weeks. Thus, it can be assumed that the study subjects were in the phase of persistent IgG levels, which may be the reason why the interval was not a statistically significant predictor of seropositivity.

Although epidemiological data document the effect of sex, age and obesity on the course of COVID-19, most studies showed no association between antibody response and age or sex, corresponding to our results (4, 23). In literature, we can find heterogeneous data on the association of body weight, specifically BMI with IgG levels detected after COVID-19. A positive correlation was noted between BMI and IgG levels (r = 0.37, p < 0.001) in the study by Racine-Brzostek et al among 1,055 individuals with metabolic syndrome, who underwent antibody analysis on average 40 days after symptom onset (24). Also, a positive correlation between BMI and IgG values (r = 0.17, p = 0.002) was revealed by the study of Shields et al on a sample of 442 HCWs with seropositivity also detected on average 40 days after the onset of COVID-19 symptoms (25). The same value of the correlation coefficient also with statistical significance was noted in our work.

While a large proportion of available studies dealt with hospitalized patients and included severe cases (4), the present study looked at the patients who, on the other hand, were mostly treated only remotely (by telephone) as outpatients, and whose course of COVID-19 was mild or moderate. Data on the distribution of disease severity vary depending on the population studied and the definition used. About 80 % of symptomatic individuals of the general population show a mild course of the disease and up to 20 % of symptomatic individuals suffer from a more severe form of the disease, according to the study by Wu et al on 72,314 Chinese and the study by Suh et al on 161 Koreans (26, 27). In our study, a mild course of COVID-19 was found in 72 % of the subjects. It is possible that a slightly higher incidence of moderate COVID-19 may have been caused by the bigger initial infectious inoculum, which is more likely in HCWs, when contracting the infection (28), as well as by the physical and mental exhaustion that occurs in HCWs after months of the pandemic.

The limitation of this study was that it was not based on a random sample of subjects of the general population after COVID-19. Recruitment of probands from HCWs having their disease recognized

as an OD may have affected the average age and the proportion of different disease courses in the study sample, which is a limitation for comparisons with the general population. After the expansion of the pilot study sample, it will be possible to specify in more detail the proportion of the subjects with persistent seropositivity, especially after longer time periods have lapsed since the acute phase of the disease.

Conclusion

Only one statistically significant predictor of persistent seropositivity of anti-spike IgG after COVID-19 was found in a pilot sample of HCWs, namely symptom duration. With each day of symptom duration, the probability of IgG seropositivity increased from 1.078 to 1.092 times ($p < 0.05$). If acute symptoms lasted longer than 17 days, the majority (almost 80 %) of the subjects maintained seropositivity in the following months. Thus, the presence of antibody immunity can be assumed from anamnestic data, which can be used in the development of vaccination strategies (for those having suffered from COVID-19) and other anti-epidemic measures.

Learning points

- Antibody responses after COVID-19 show significant differences among convalescents
- We investigated the existence of readily available predictors of seropositivity
- 152 healthcare workers were included approximately 2 months after the disease onset
- Only symptom duration was a significant predictor of seropositivity
- Symptom duration exceeding 17 days predicted seropositivity in majority of the subjects

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