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Prevalence and duration of anti-SARS-CoV-2 antibodies in healthcare workers

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ABSTRACT

Introduction. Knowledge of the seroprevalence and duration of antibodies against SARS-CoV-2 was needed in the early phases of the COVID-19 pandemic and is still necessary for policy makers and healthcare professionals. This information allows us to better understand the risk of reinfection in previously infected individuals.

Methods. We investigated the prevalence and duration of detectable antibodies against SARS-CoV-2 in sequentially collected samples from 379 healthcare professionals.

Results. SARS-CoV-2 seroprevalence at inclusion was 5.3% (95% confidence interval (CI): 3.3-8.0%) and 25% of seropositive participants reverted during follow-up. At the end of follow-up, the calculated probability of having detectable antibodies among former seropositive participants was 72.2% (95% CI: 54.2-96.2%).

Conclusion. Antibodies against SARS-CoV-2 were detectable in a subset of infected individuals for a minimum of 39 weeks.

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Trial registration. The study was registered with the Danish National Committee on Health Research Ethics (H-20022312).

Healthcare professionals were at the core of society's response towards the COVID-19 pandemic and were at high risk of becoming infected in the early pandemic phase [1]. The rate of infections among healthcare professionals, work absence from COVID-19 and the need to self-isolate directly impact healthcare capacity. The consensus view is that detection of anti-SARS-CoV-2 antibodies represents a reliable marker of past infection as more than 96% of infected individuals become seropositive two to three weeks after infection [2]. To investigate the duration of SARS-CoV-2 antibodies among healthcare professionals in the early stages of the COVID-19 pandemic, when the Wuhan strain of the virus was dominant, we determined the seroprevalence and monitored the duration of SARS-CoV-2 antibodies in samples collected from staff at two Danish acute-care hospitals between March and December, 2020.

METHODS

Study design

A prospective observational cohort study was initiated in March 2020 with two acute-care hospitals as study sites; Nordsjællands Hospital and Nykøbing Falster Sygehus, both located in Denmark. Participants were recruited through contact with department managers to ensure rapid recruitment while the epidemic was still in its early phase of staff with and without patient contact and representing different professions. A total of 379 healthcare professionals provided blood samples for SARS-CoV-2 antibody analysis during the initial 12-week inclusion period. The participants also contributed information regarding demographic and health characteristics along with information on symptoms since the previous visit. Samples were taken weekly or bi-weekly according to the study plan, which was independent of the timing of any symptoms. Any sampling that coincided with symptoms was cancelled because regulations prescribed isolation (**Table 1**). Participants' serum samples were tested for antibodies against SARS-CoV-2 using three serological methods. A direct ELISA measuring immunoglobulin (Ig)M, IgG and IgA against the SARS-CoV-2 receptor-binding domain (RBD) on the spike protein; a RBD sandwich ELISA measuring total antibodies against the SARS-CoV-2 RBD as described by Hansen et al. [3]; and a pseudotype microneutralisation assay, as described by Thompson et al. [4]. The assays had the spike glycoprotein as their target owing to its uniqueness to SARS-CoV-2 compared with other coronaviruses. Measurements were performed and the assays manufactured at the Department of Clinical Immunology at Rigshospitalet in Copenhagen, Denmark, and at the Department of Zoology at the University of Oxford, UK.

TABLE 1 Participant characteristics, self-reported.

	n (%)	Seropositive, % (95% CI)	Mean ± SD
Study site			
NOH	231 (60.9)		
NFS	148 (39.1)		
All	379 (100.0)	5.3 (3.3-8.0)	
Age, yrs			45.56 ± 11.28
Gender			
Male	73 (19.3)	1.4 (0.0-7.4)	
Female	306 (80.7)	6.2 (3.8;9.5)	
Occupation			
Medical doctor	114 (30.1)	2.6 (0.1-7.5)	
Nurse	143 (37.7)	7.7 (3.9-13.3)	
Healthcare assistant	35 (9.2)	8.6 (1.8-23.1)	
Other[a]	86 (22.7)	3.5 (0.7-9.8)	
Chronic conditions before the start of the study, n			
0-1	354 (93.4)	4.8 (2.8-7.6)	
≥ 2	25 (6.6)	12.0 (2.6-31.2)	
Smoking			
Non-smoker	264 (69.7)	4.5 (2.4-7.8)	
Ex-smoker	67 (17.7)	7.5 (2.5-16.6)	
Smoker	47 (12.4)	6.4 (1.3-17.5)	
BMI, kg/m²			
Normal weight: < 25	218 (57.8)	4.6 (2.2-8.3)	
Overweight: [25-30 [	107 (28.4)	8.4 (3.9-15.4)	
Obese: ≥ 30	52 (13.8)	1.9 (0.0-10.3)	
Respiratory symptoms?			
No	223 (60.7)	2.24 (0.7-5.2)	
Yes	156 (39.3)	9.6 (5.5-15.4)	

CI = confidence interval: NFS = Nykøbing Falster Sygehus; NOH = Nordsjællands Hospital; SD = standard deviation.
a) Bioanalyst, midwife, dietitian, medical secretary, consultant, administrative employee, radiologist, pharmacologist, physiotherapist, project employee.

Detectable antibodies were defined as antibodies documented with any of the utilized methods as there was no indication of a significant performance differences between them in our study when the same samples were analysed with two or more of the methods used. All three methods were used throughout the study period. After the inclusion period, participants with detectable SARS-CoV-2 antibodies were invited to provide new blood samples three months after the end of the inclusion period (study week 28) and again after two additional months had passed (study week 39).

Statistical analyses

We applied survival analysis calculating the survival function of detectable antibodies at the end of follow-up. A

Kaplan-Meier plot was drawn to illustrate the function. Data were analysed in R version 3.6.1. The packages ”survival” [5], ”prodlim” [6] and ”survminer” [7] were used for survival analysis.

Trial registration: The study was registered with the Danish National Committee on Health Research Ethics (H-20022312).

RESULTS

A total of 2,536 blood samples were collected from 379 participants during the 12-week inclusion period from 30 March to 29 June 2020. Blood samples were drawn weekly or bi-weekly during this period. Of the 379 participants included, 276 (72.8%) completed the entire study period and 199 (52.5%) participants completed all visits.

The overall seroprevalence recorded during the initial study period was 5.3% (95% confidence interval (CI): 3.3-8.0%). On initial testing, nine participants had detectable antibodies against SARS-CoV-2, rising to 20 after 12 weeks of follow-up. Where participants reported symptoms, the mean time from symptoms to a positive test was one week (data not shown), with participants both reporting symptoms before and after detection of antibodies. The 20 seropositive participants were invited for follow-up testing; of these, 11 were tested at study week 28 and 16 at study week 39. Overall, 18 participants (90% of the seropositives) provided samples during follow-up (**Figure 1**). The methods of serology used in the analysis had a similar performance with small variations during the follow-up period (**Figure 2**). The final rate of detectable antibodies at week 39 was 81%, with seroreversion observed in two of 11 subjects at week 28 and in three of 16 samples at study week 39.

FIGURE 1 Rate of detectable antibodies across study weeks among seropositive participants during initial 12-week inclusion period (dotted line) and follow-up (solid line).

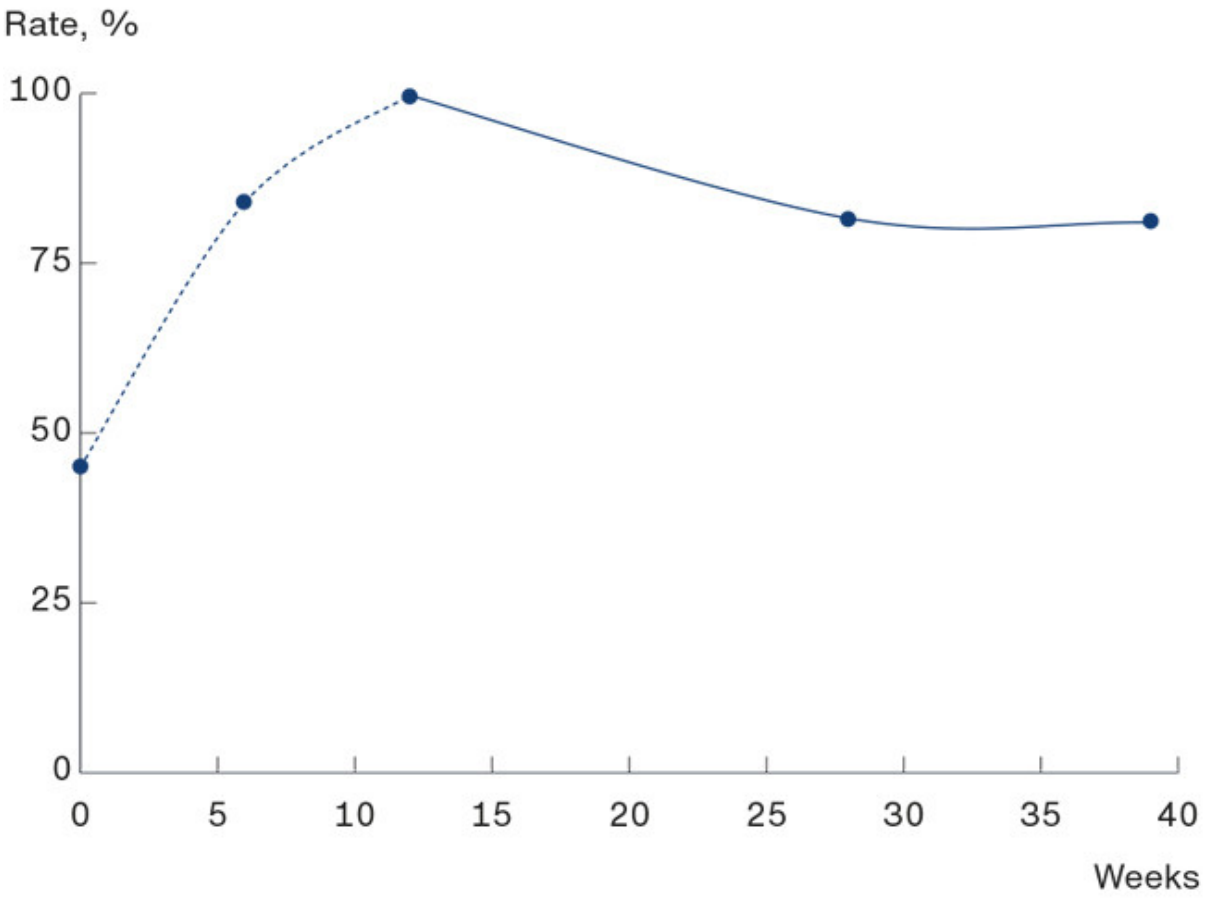
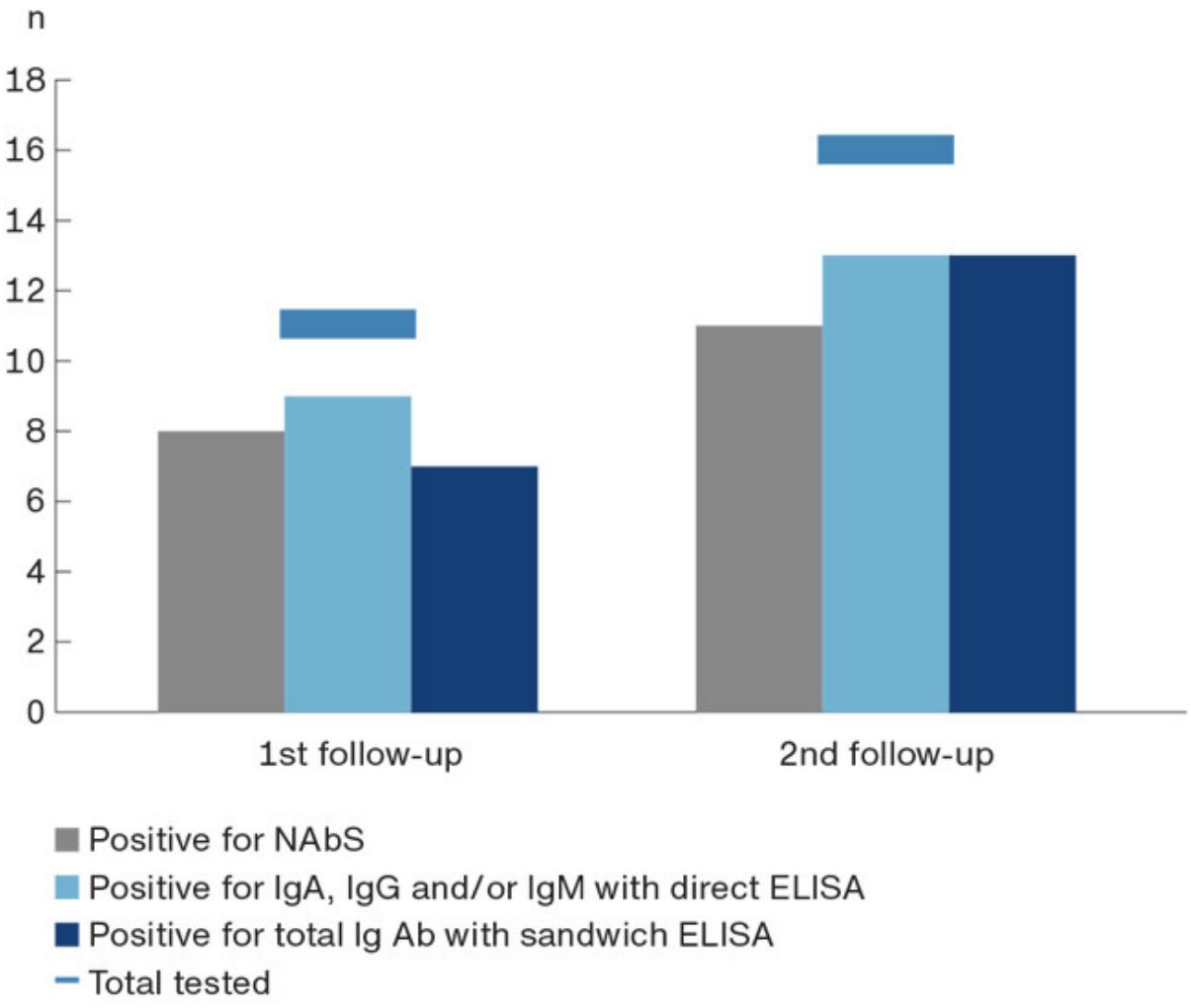


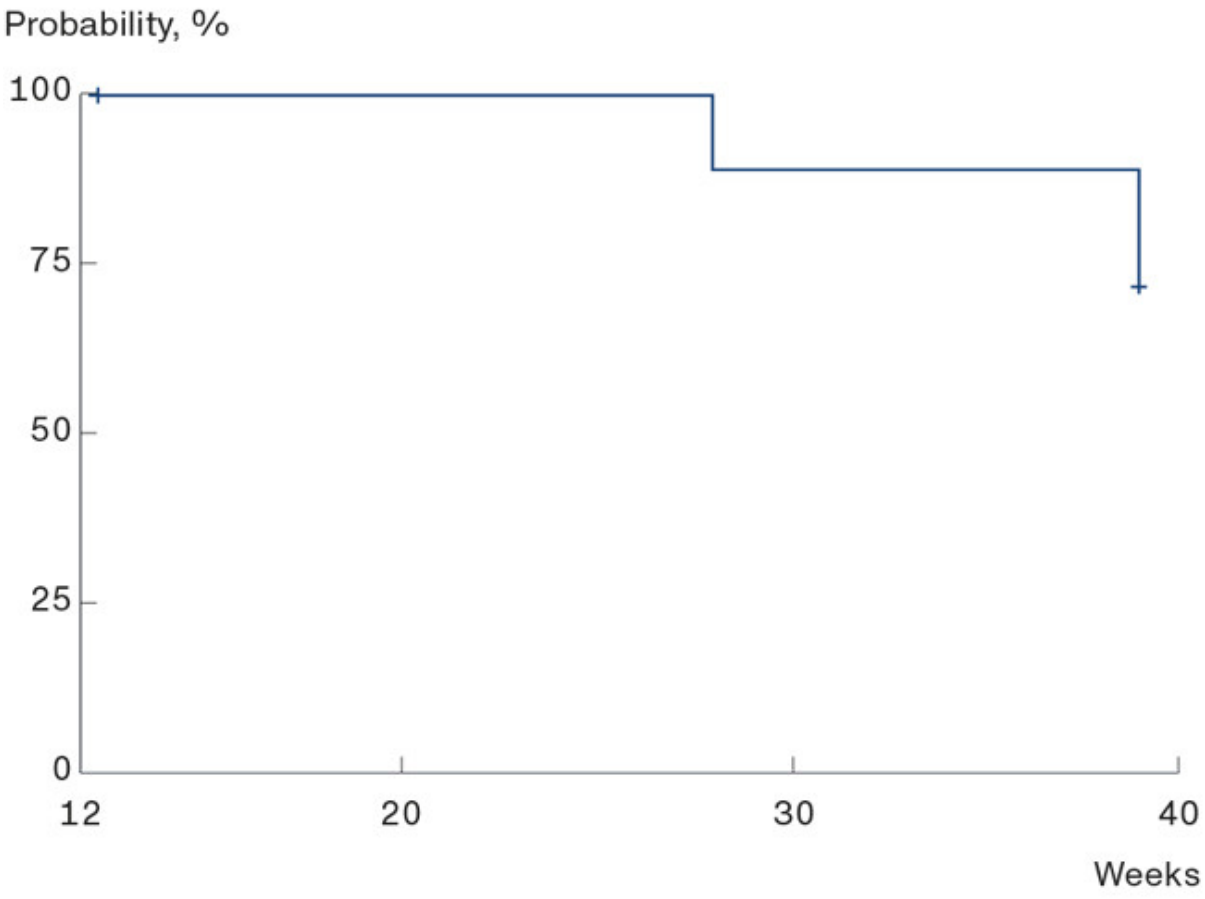
FIGURE 2 Number of samples with detectable antibodies in three assays during follow-up among seropositive participants.



ELISA = enzyme-linked immunosorbent assay; Ig = immunoglobulin; NAbs = neutralising antibodies.

To investigate the effects of loss to follow-up and multiple follow-up measures of antibody detectability, we applied a survival analysis framework to investigate the duration of detectable antibodies in our cohort. We investigated the probability of having detectable antibodies over time, represented by study weeks. At the end of follow-up in study week 39, our survival analysis showed a probability of detectable antibodies of 72% (95% CI: 54-96.2%) (**Figure 3**).

FIGURE 3 Kaplan-Meier curve showing the probability of detectable antibodies over time among seropositive participants at inclusion.



DISCUSSION

Among the healthcare professionals with detectable antibodies during inclusion, most were followed for five additional months with a high rate of antibody detection at the end of follow-up. When loss to follow-up was considered, the probability of antibody detection remained high.

At the onset of the pandemic, no formal guidance was available on protective equipment, e.g. masks and hazmat suits for healthcare professionals, though guidelines and instructions for these were implemented during the initial study period. Also, while symptomatic citizens were tested throughout the period, no consensus existed on how or whether widespread testing for infection could be established for large-scale testing of asymptomatic individuals in Denmark. Therefore, the healthcare professionals working at acute-care hospitals in Denmark at the onset of the pandemic had a high risk of exposure to SARS-CoV-2 but lacked the means to detect their infection status unless they developed COVID-related symptoms.

With 379 participants originally included in the study during a period with increasing numbers of infections, we expected 10-15% of participants to have detectable antibodies after 12 weeks. As 5.3% of the participants had detectable antibodies, the sample size of our follow-up population (n = 20) was smaller than anticipated. In April

2020, during our inclusion period, a Danish study utilizing point-of-care tests for IgM and IgG antibodies against SARS-CoV-2 found antibodies in 4.04% (95% CI: 3.82-4.27%) of 28,792 healthcare professionals [1]. Our rate of detectable antibodies among our 379 initial participants was in line with these findings.

It is well known that antibodies against other communicable viral diseases last for different periods of time, ranging from two years to lifelong seropositivity [8, 9]. As a new disease, very little was known or could be inferred about the durability of antibodies against SARS-CoV-2 following infection. Our study contributes to the body of knowledge in this field.

Studies have already shown that SARS-CoV-2 antibody duration varies between individuals. One study found a rapid decline in detectable antibodies in COVID-19 patients up to 90 days after infection [10]. Another study found that 20 participants had stable titers of antibodies up to 105 days after infection [11]. Recently, Dehgani-Mobaraki et al. showed that antibodies were detectable in 96.8% of 32 recovered COVID-19 patients in Italy 14 months after infection [12]. Zeng et al. presented a one-year IgG prevalence of 82.9% in a cohort of 538 confirmed COVID-19 cases from Wuhan [13]. Our study was different from these studies with respect to study design, methods of serology and participant characteristics. Both Zeng and Dehgani-Mobaraki included former patients with confirmed or symptomatic disease, whereas we recruited subjects based on their antibody status during inclusion. It has been shown that development and retainment of antibodies against SARS-CoV-2 is related to disease severity and symptoms as well as to the method of testing [2].

We did not investigate what demographic or clinical attributes in the study subjects were associated with seroreversion. However, Zeng et al. showed a lower retention of antibodies in younger individuals one year after infection, whereas loss of sense of taste and smell was associated with antibody detectability after 14 months in the study by Dehgani-Mobaraki et al. [12, 13]. Hansen et al. showed a correlation between disease severity and antibody response [3]. We suggest further analysis of risk factors associated with different durations of antibodies against SARS-CoV-2 where and when larger study populations are available. Our study population consisted of working adults in hospitals who were not fully representative of the general population with respect to their age range or general health. We therefore suggest broadening the study population and/or using national level data in future investigations exploring the duration of detectable antibodies.

CONCLUSION

We found detectable anti-SARS-CoV-2 antibodies in a small proportion of our study population during the recruitment period of the study. When investigating the duration of detectable antibodies within this subpopulation after 28 and 39 weeks, we found a measurable proportion of individual who lost detectable antibodies over the study period. Our findings revealed stability of detectable antibodies over time. Though we showed that not all individuals with antibodies after infection have lasting detectable antibody levels, we also showed that antibodies against SARS-CoV-2 were detectable in a subset of infected individuals for a minimum of 39 weeks.

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Conflicts of interes Potential conflicts of interest have been declared. Disclosure forms provided by the authors are available with the article at ugeskriftet.dk/dmj

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REFERENCES

1. Iversen K, Bundgaard H, Hasselbalch RB et al. Risk of COVID-19 in healthcare workers in Denmark: an observational cohort study. Lancet Infect Dis. 2020;20(12):1401-8.
2. Harritshøj LH, Gybel-Brask M, Afzal S et al. Comparison of 16 serological SARS-CoV-2 immunoassays in 16 clinical laboratories. J Clin Microbiol. 2021;59(5):e02596-20.
3. Hansen CB, Jarlhelt I, Pérez-Alós L et al. SARS-CoV-2 antibody responses are correlated to disease severity in COVID-19 convalescent individuals. J Immunol. 2021;206(1):109-17.
4. Thompson CP, Grayson NE, Paton RS et al. Detection of neutralising antibodies to SARS-CoV-2 to determine population exposure in Scottish blood donors between March and May 2020. Euro Surveill. 2020;25(42):2000685.
5. Therneau TM. A Package for Survival Analysis in R. https://CRAN.R-project.org/package=survival (2020).
6. Gerds TA. prodlim: Product-Limit Estimation for Censored Event History Analysis. R package version 2019.11.13. https://CRAN.R-project.org/package=prodlim (2019).
7. Kassambara A, Kosinski M, Biecek P. survminer: Drawing Survival Curves using 'ggplot2'. R package version 0.4.9. https://CRAN.R-project.org/package=survminer (2021).
8. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med. 2007;357(19):1903-15.
9. Barclay WS, al-Nakib W, Higgins PG, Tyrrell DA. The time course of the humoral immune response to rhinovirus infection. Epidemiol Infect. 1989;103(3):659-69.
10. Ibarrondo FJ, Fulcher JA, Goodman-Meza D et al. Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. N Engl J Med. 2020;383(11):1085-7.
11. Isho B, Abe KT, Zuo M et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol. 2020;5(52):eabe5511.
12. Dehgani-Mobaraki P, Zaidi AK, Yadav N et al. Longitudinal observation of antibody responses for 14 months after SARS-CoV-2 infection. Clin Immunol. 2021;230:108814.
13. Zeng F, Wu M, Wang J et al. Over 1-year duration and age difference of SARS-CoV-2 antibodies in convalescent COVID-19 patients. J Med Virol. 2021;93(12):6506-11.