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Evaluation of Serum Antibody Levels and Presence of Neutralizing Antibodies in Patients with Mild and Moderate Coronavirus Disease-2019

Hafif ve Orta Şiddetli Koronavirüs Hastalığı-2019 Hastalarında Serum Antikor Düzeylerinin ve Nötralizan Antikor Varlığının Değerlendirilmesi

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Abstract

Introduction: Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) antibodies are produced in persons who have been infected by the virus or have received the vaccine. Many features of these antibodies, including their dynamics and neutralization capacities, are still unclear. Understanding the immune response of the host is very important for the development of appropriate treatment methods, vaccines, and epidemiological control strategies. The present study aimed to monitor the change in antibody levels over time in individuals diagnosed with SARS-CoV-2 infections and to determine their neutralization capacity.
Materials and Methods: Anti-nucleocapsid and anti-spike antibody titers were measured using different kits on monthly obtained serum samples of patients of patients with SARS-CoV-2 infection. The neutralizing antibodies were evaluated using a microneutralization assay.
Results: A total of 134 serum samples taken from 43 patients with a mild-moderate disease course were analyzed. Anti-spike antibody positivity was detected on day seven at the earliest and day 334 at the latest following a positive polymerase chain reaction (PCR) test. The mean antibody levels were observed to increase gradually to a peak after three months, and then started to decrease after month six. Anti-nucleocapsid IgM and IgG antibodies were detected alone or in combination. The highest neutralizing antibody titer was 1/80 in the first month, which was seen to drop below 1/10 after four months.
Conclusion: The combined use of kits for the detection of antibodies against different antigens or testing total antibodies would result in a more accurate and earlier detection of the antibodies that start to emerge on the seventh day and decrease six months after SARS-CoV-2 PCR positivity. In addition, the dramatic decrease in neutralizing antibody titers after four months may be one of the causes of early reinfections.
Keywords: Coronavirus disease 2019, anti-SARS-CoV-2 antibody, neutralizing antibody

Öz

Giriş: Şiddetli akut solunum yolu sendromu-Koronavirüs-2 (SARS-CoV-2) antikorları, virüsle enfekte olmuş veya aşısını olmuş kişilerde oluşur. Bu antikorların dinamikleri, nötralizasyon kapasiteleri gibi birçok özelliği hala netliğe kavuşmamıştır. Konağın immün yanıtının anlaşılması; uygun tedavi yöntemlerinin, aşıların ve epidemiyolojik kontrol stratejilerinin geliştirilmesi için oldukça önemlidir. Bu çalışma, SARS-CoV-2 enfeksiyonu teşhisi konan bireylerde antikor düzeylerinin zaman içindeki değişimini izlemeyi ve nötralizasyon kapasitelerini belirlemeyi amaçlamaktadır.
Gereç ve Yöntem: SARS-CoV-2 enfeksiyonlu hastalardan aylık olarak alınan serum örneklerinde farklı kitler kullanılarak anti-nükleokapsid ve anti-spike antikor titreleri ölçüldü. Nötralize edici antikorlar, mikronötralizasyon yöntemi ile belirlendi.
Bulgular: Hafif-orta dereceli hastalık seyri olan 43 hastadan alınan toplam 134 serum örneği analiz edildi. Anti-spike antikor pozitifliği, pozitif polimeraz zincir reaksiyonu testinin ardından en erken yedinci günde, en geç 334. günde tespit edildi. Ortalama antikor düzeylerinin üçüncü aydan

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Öz

sonra kademeli olarak yükseldiği ve altıncı aydan sonra azalmaya başladığı gözlendi. Anti-nükleokapsid IgM ve IgG antikorları tek başına veya kombinasyon halinde tespit edildi. En yüksek nötralize edici antikor titresi ilk ayda 1/80 iken, dört ay sonra 1/10'un altına düştüğü belirlendi.

Sonuç: Farklı antijenlere karşı antikorların saptanması veya total antikorların test edilmesi için kitlerin birlikte kullanılması, yedinci günde ortaya çıkmaya başlayan ve SARS-CoV'dan altı ay sonra azalan antikorların daha doğru ve daha erken saptanmasını sağlayacaktır. Ayrıca nötralizan antikor titrelerinin dört ay sonra dramatik olarak azalması, erken reenfeksiyonların nedenlerinden biri olabilir.

Anahtar Kelimeler: Koronavirüs hastalığı-2019, anti-SARS-CoV-2 antikoru, nötralizan antikor

Introduction

Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2), the causative agent of Coronavirus disease-2019 (COVID-19) disease that was declared a global pandemic on March 11, 2020 by the World Health Organization (WHO), is a Coronavirus that first emerged in December 2019 in the People's Republic of China and spread rapidly worldwide[1]. The SARS-CoV-2 genome is approximately 30 kb and encodes 27 proteins including eight helper proteins and 15 non-structural proteins[2]. Viral structural proteins include spike glycoproteins, membrane glycoproteins, envelope glycoproteins, and nucleocapsid phosphoproteins[2]. Trimeric S protein is known to be the first viral fraction to bind to the host cell receptors through the receptor binding domain and N-terminal domain of the S1 subunit, while the S2 subunit is known to aid in the fusion of the virus and the host cell membrane[2,3]. On the other hand, N protein is the structural compound of the nucleocapsid and has important functions in disease pathogenesis, virus multiplication, and RNA packaging[4].

Severe acute respiratory syndrome-Coronavirus-2 antibodies are produced in persons who have been infected by the virus or who have received the vaccine. Some of the antibodies that develop against the spike and nucleocapsid proteins have a neutralizing character[5]. Neutralizing antibodies (NAbs) prevent virus entry into the cell, fusion with the cell, or release from the cell, thereby preventing the development and spread of infection[6]. Tests based on chemiluminescence immunoassay, enzyme-linked-immunosorbent assay, and lateral flow immunoassay (LFIA) are frequently used in the laboratory diagnoses of COVID-19. These detect mainly viral structural proteins or the specific antibodies that develop against these proteins in whole blood or serum[5].

Most commercial diagnostic kits detect antibodies against the spike and nucleocapsid proteins[5]. Many features of the anti-SARS-CoV-2 antibodies, such as their dynamics and neutralization capacities, are still unclear, although understanding the immune response of the host is important for the development of appropriate treatment methods, vaccines, and epidemiological control strategies[7].

This study aimed to monitor the change over time of the antibody levels in individuals diagnosed with COVID-19 and to determine their neutralization capacity.

Materials and Methods

Study Group

The study group comprised individuals identified with a SARS-CoV-2 infection between March 2020 and April 2021 through a positive virus RNA identified from nasopharyngeal swab samples using the real-time polymerase chain reaction (RT-PCR) method. Serum samples obtained monthly, if possible, from the study participants, starting from the date of SARS-CoV-2 RT-PCR test positivity, were stored at -80 °C after aliquoting. Participants who were reinfected or vaccinated against SARS-CoV-2 in the follow-up period were excluded from the study. All the patients or their legal guardians signed the informed consent form.

Detection of SARS-CoV-2 RNA

The SARS-CoV-2 genomic RNA was detected in the nasopharyngeal samples of patients with COVID-19 infection using a Bio-speedy SARS-CoV-2 (2019-nCoV) RT-qPCR detection kit (Bioeksen, İstanbul, Turkey) on a LightCycler® 480 Instrument II (Roche Molecular Systems, USA) using the reverse transcription RT-PCR method.

Detection of Anti-SARS-CoV-2 Antibodies

Electrochemiluminescent Immunoassay

The total anti-nucleocapsid (anti-N) antibody levels were measured in serum samples using the Elecsys anti-SARS-CoV-2 (Roche Diagnostics International AG, Rotkreuz, Switzerland) kit. Assays with an anti-N antibody level <1.0 cut off index (COI) and ≥1.0 COI were considered non-reactive and reactive, respectively.

The total anti-spike (anti-S) antibody levels were determined in samples using the Elecsys anti-SARS-CoV-2 S Quantitative (Roche Diagnostics International AG, Rotkreuz, Switzerland) kit. Assays with an anti-S antibody level of <0.80 U/ml and ≥0.80 U/ml were considered non-reactive and reactive, respectively.

Lateral Flow Immunoassay

Presence of IgG and IgM antibodies against the nucleocapsid protein was determined in randomly selected anti-N positive samples [tested with electrochemiluminescent immunoassay (ECLIA)] using a SARS-CoV-2 rapid antibody test (Roche Diagnostics International AG, Rotkreuz, Switzerland) kit.

In vitro Microneutralization Assay for SARS-CoV-2

The previously described virus neutralization assay procedures were performed[8,9].

Cells and the Virus

Vero E6 (ATCC CRL-1586 Cercopithecus aethiops kidney epithelial cells) cells were cultured using a medium including 10% fetal bovine serum at 37 °C in a 5% CO_2 atmosphere.

The studied SARS-CoV-2 virus is a clinical isolate, the full genome sequence of which is registered in the NCBI GenBank NIH Genetic Sequence Database (GenBank: MT955161.1).

Microneutralization Assay

The serum samples were incubated at 56 °C for 30 min for complement inactivation. The serum samples were diluted two-fold starting at 1/10 (1/10-1/640) with a maintenance medium in 96-well cell culture plates and subsequently incubated for 15 min at 36±0.5 °C in a 5% CO_2 atmosphere. A virus suspension including 100 $TCID_{50}$ (median tissue culture infectious dose) was then added at a 1:1 ratio on top of each serum dilution, and the plates were subsequently incubated at 36±0.5 °C in a 5% CO_2 atmosphere for 60 min.

Following incubation, 100 µl of Vero E6 cell suspension prepared in 2×10^5 cells/ml quantities was added to each well, and the plates were subsequently incubated for four days at 36±0.5 °C in a 5% CO_2 atmosphere. The cytopathic effect of each well was assessed under a microscope and recorded, and the neutralizing titer was calculated based on the dilution number of 50% protective condition.

Statistical Analysis

The obtained data were analyzed using IBM Statistical Package for the Social Sciences Statistics version 23.0 (IBM Corp. Armonk. N.Y. USA). A chi-square test or a Fisher's exact test were used for the comparison of categorical variables, and a Mann-Whitney U test was used for the comparison of non-normally distributed continuous variables. The threshold level (p value) for statistical significance was accepted as <0.05.

Results

A total of 134 serum samples collected from 43 patients who tested positive for SARS-CoV-2 in a PCR test based on nasopharyngeal swab samples and whose disease course was mild-moderate according to the WHO criteria were analyzed in the present study[5]. The number of serum samples decreased in the later months of the study, as those reinfected or vaccinated against SARS-CoV-2 during the follow-up period were excluded from the study. All patients were treated with favipiravir, and none of them received anti-inflammatory or corticosteroid therapy.

The mean age of the patients was 39.5±2.1 years, and the median age was 35 years (minimum-maximum: three months-68 years); 58.1% were female and 41.9% were male. No statistically significant difference was found in the distribution of antibody positivity in terms of age or gender (p>0.05).

The results of the antibody tests against the spike and nucleocapsid proteins of SARS-CoV-2 based on the time elapsed since the recording of a positive RT-PCR test based on nasopharyngeal swab samples are presented in Tables 1 and 2 and Figure 1.

The first anti-S antibody was identified in a patient on day seven following PCR positivity at a level of 4.49 U/ml and in another patient at the latest on day 334 at a level of 11.14 U/ml.

Antibody titers showed a gradual increase to reach their maximum level after month three, and declined starting from month six. No anti-N antibodies were detected in five patients during the first month, while anti-S antibodies were detected. In one patient, no antibodies were detected until three months.

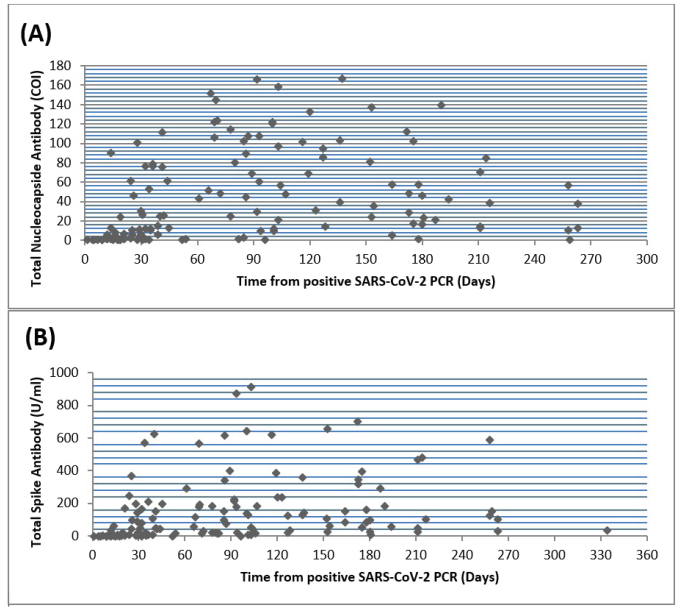

Figure 1. A) Total anti-nucleocapsid antibody levels of tested electrochemiluminescent immunoassay according to time from positive SARS-CoV-2 PCR (days); B) Total anti-spike antibody levels of tested electrochemiluminescent immunoassay according to time from positive SARS-CoV-2 PCR (days)

SARS-CoV-2: Severe acute respiratory syndrome-Coronavirus-2, PCR: Polymerase chain reaction

Moreover, in one patient, anti-N was determined to be positive at a level of 1.34 COI in the serum sample obtained on day 12, while anti-S (0.419 U/ml) was found to be negative. No antibodies were detected in the two serum samples collected on days 52 and 96 in one patient.

Anti-N antibodies (IgM and IgG) were investigated, either alone or combined, starting on day 14 and continuing up to day 263 after PCR positivity; using LFIA, IgG positivity was ≥80% in all periods in which analyses were carried out, while the highest rate of IgM positivity was identified as 86.7% in the first month. The IgG and IgM antibody levels against nucleocapsid proteins are presented in Table 2.

The NAb titers were highest in the samples taken during the first moth (1/80) and dropped to below 1/10 titers after month four in those with mild-moderate disease. The results of the NAbs detected in the present study over time following PCR positivity is presented in Table 3 and Figure 2.

Discussion

The clinical picture of patients with COVID-19 covers a wide spectrum, including asymptomatic cases and severe cases with septic shock and multi-organ failure[10]. Previous studies have suggested that this diversity in the clinical picture might be attributable to the involvement of the congenital and acquired immune system in the pathogenesis of COVID-19[10], which suggests that the adaptive immune response of the host should be analyzed in detail prior to the launch of antiviral treatment, vaccines, or epidemiological control strategies against COVID-19, for which there is as yet no specific antiviral treatment[11]. Knowledge of SARS-CoV-2 serology and the neutralizing capacity of developing antibodies has not yet been extensively validated. Strategies need to be developed to better understand the antigen-antibody relationships detected by conventional immunoassays[12]. When serological tests are evaluated together with tests that reveal the neutralization capacity, they provide important contributions in determining the protective immunity that develops against viral infections in individuals[13].

The disease severity and the mortality rate are known to be related to the high rate of comorbidities, the increased proinflammatory response, and the decreased antiviral cytokine levels of elderly patients who contract COVID-19[10,14]. Disease severity is reported to be more severe in the male gender due to the involvement of sex hormones in the inflammatory processes, the angiotensin-converting enzyme-2 expression level, and lifestyle differences[14]. In the present study, the median age of the patient sample was 35 years, and no statistically significant difference was found in the monthly distribution of antibody positivity in terms of either the age group or gender.

Studies of the seropositivity of the antibodies that develop against SARS-CoV-2 are ongoing. Lee et al.[15] reported detecting

Table 1. Results of tested electrochemiluminescent immunoassay according to time from positive SARS-CoV-2 PCR (days)

Antibody type	Time from positive SARS-CoV-2 PCR (days)	Number of tested sample (n)	Seropositivity n (%)	Mean±SD	Range (min-max)	Median	IQR (per 25-75)
Total anti-nucleocapsid antibody (COI)		131	109 (83.2)		0.1-199.7		
	0-30	40	27 (67.5)	12.3±23.2	0.1-100.1	3.2	0.3-11.0
	30-60	18	13 (72.2)	32.2±36.0	0.1-111.1	13.7	0.8-76.0
	60-90	19	18 (94.7)	78.3±45.0	0.9-151.7	80.5	44.9-114.4
	90-120	19	18 (94.7)	78.1±56.6	0.1-166.9	68.1	21.6-12.8
	120-150	6	6 (100)	61.3±37.8	14.1-103.2	62.4	26.6-97.1
	150-180	15	14 (93.3)	51.2±40.7	0.7-136.5	45.4	17.3-80.8
	>180	14	13 (92.9)	40.3±37.7	0.3-139.7	29.9	13.2-60.3
Total anti-spike antibody (U/ml)		134	122 (91.0)		0.4-909.8		
	0-30	40	34 (85.0)	46.9±81.0	0.4-366.8	6.45	1.9-59.4
	30-60	18	16 (88.9)	116.2±187.8	0.4-623.2	28.41	7.1-161.8
	60-90	20	20 (100)	170.3±182.6	14.6-614.9	104.87	24.6-265.7
	90-120	20	19 (95.0)	250.9±285.6	0.4-909.8	158.40	25.8-349.4
	120-150	6	6 (100)	221.5±129.0	21.7-361.9	124.8	33.2-269.4
	150-180	15	15 (100)	217.4±220.1	23.8-700.7	107.2	64.2-341.80
	>180	15	15 (100)	179.0±188.2	11.6-586.6	103.5	34.1-289.2

IQR: Interquartile range, COI: Cut-off index, U/ml: Unit/mililiter, min-max: Minimum-maximum, SARS-CoV-2: Severe acute respiratory syndrome-Coronavirus-2, PCR: Polymerase chain reaction

Table 2. Results of tested lateral flow immunoassay according to time from positive SARS-CoV-2 PCR (days)

Antibody type	Time from positive SARS-CoV-2 PCR (days)	Number of tested	Seropositivty IgM n (%)	Seropositivty IgG n (%)
Anti-nucleocapsid antibody		79	37 (46.8)	76 (96.2)
	0-30	15	13 (86.7)	14 (93.3)
	30-60	16	9 (56.3)	15 (93.8)
	60-90	15	7 (46.7)	15 (100.0)
	90-120	14	4 (28.6)	14 (100.0
	120-150	5	0 (0)	4 (80.0)
	150-180	6	2 (33.3)	6 (100.0)
	>180	8	2 (25)	8 (100.0)

SARS-CoV-2: Severe acute respiratory syndrome-Coronavirus-2, PCR: Polymerase chain reaction, IgM: Immunoglobulin M, IgG: Immunoglobulin G

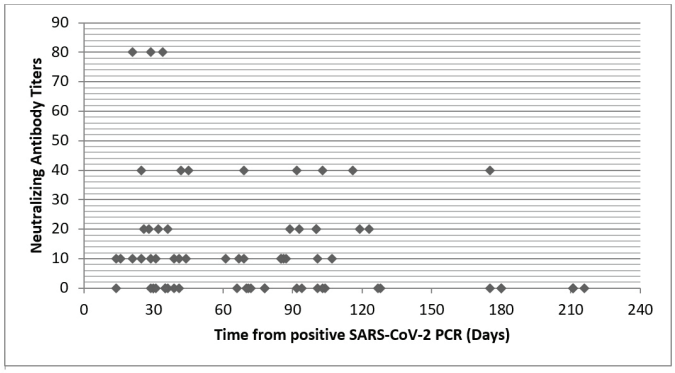

Figure 2. Results of tested in vitro microneutralization assay according to time from positive SARS-CoV-2 PCR (days)

SARS-CoV-2: Severe acute respiratory syndrome-Coronavirus-2, PCR: Polymerase chain reaction

both anti-N IgM and IgG antibodies at the earliest on day five, while Wang et al.[16] reported on the presence of IgG antibodies at the earliest on day seven but without differentiating their type. Previous studies have reported the presence of anti-S antibodies up to 12 months[17]. In this study, serological tests were positive on the seventh day at the earliest and the 334th day at the latest (anti-S antibody), and it was observed that the antibody response in SARS-CoV-2 infection was the same as in other acute viral infections[18-20]. On the other hand, approximately 5% of the population with the disease was reported to be seronegative[21]. In this study, in one patient, antibodies could not be detected until three months after PCR positivity, and this patient was considered to be at risk of severe reinfection.

Nucleocapsid and spike antigens with high immunogenic properties have been frequently used for the serological diagnosis of COVID-19, and there are studies with different findings on this subject[22]. Van Elslande et al.[23] reported that anti-N antibodies develop earlier than anti-S antibodies and result in fewer false positives because it is more conserved. However, To et al.[24] reported detecting anti-S antibodies earlier than anti-N antibodies. Anti-S IgG levels were reported to begin to decrease two-three months after the initiation of symptoms in a study by Long et al.[25], while Dan et al.[26] demonstrated that the half-life of the anti-S and anti-N antibodies were 140 days and 68 days, respectively, and that these antibodies retained their presence for eight months. In the present study, no anti-N antibodies were detected in the serum samples of six patients in the first month of the disease, while anti-S antibodies were detected. In one patient, anti-N antibodies were found to be positive and anti-S antibodies to be negative on day 12. Anti-N antibody levels were found to decrease three-four months after PCR positivity in patients from whom serial serum samples could be retrieved, although no sero-negativity was identified in any of the samples analyzed. This suggests that using different kits to detect the antibodies that develop against different antigens would increase the possibility of detecting antibodies when determining antibodies against SARS-CoV-2.

Another subject of interest in SARS-CoV-2 infection is investigating to what extent the type of antibody would be beneficial in determining the period of the disease. IgM type antibodies have frequently been accepted as a marker of acute infection, although IgMs do not always show up before the antibodies in the IgG type. This in turn limits the use of IgMs as a marker of acute or new infections, such SARS-CoV-2 infection[21,22,24]. Only the anti-N antibodies could be differentiated as IgG and IgM with a LFIA in the present study. The antibody seropositivity rates in the IgG type were found to be higher than in the IgM type in all periods evaluated in this present study. While the rate of IgM positivity was seen to decrease one month after PCR positivity, some patients had IgM antibodies to nucleocapsid proteins even after six months. The presence of IgM and IgG was investigated with LFIA in some samples that were found to be anti-N antibody positive by ECLIA, and IgM or IgG alone was found to be negative in some

Table 3. Results of tested *in vitro* microneutralization assay according to time from positive SARS-CoV-2 PCR (days)

Time from positive SARS-CoV-2 PCR (days)	Number of tested sample (n)	Neutrolization antibody positivity n (%)	Mean±SD	Range (min-max)	Median	IQR (per 25-75)
Total	72			0-80		
0-30	15	11 (77.3)	20.0±26.5	0-80	10.0	0-20.0
30-60	16	7 (43.75)	16.3±21.3	0-80	10.0	0-20.0
60-90	16	10 (62.5)	16.6±12.9	0-40	10.0	0-10.0
90-120	14	9 (64.3)	15.7±15.5	0-40	15.0	0-25.0
120-150	3	1 (33,3)	6.7±11.5	0-20	0	0
150-180	4	1 (25.0)	10.0±20.0	0-40	0	0-30.0
>180	4	0	0	0	0	0

IQR: Interquartile range, SARS-CoV-2: Severe acute respiratory syndrome-Coronavirus-2, PCR: Polymerase chain reaction, SD: Standard deviation

samples. Therefore, it would be appropriate to use IgM and IgG tests together if the LFIA test is to be used in the follow-up of antibody titers.

Neutralizing antibody levels were reported to be lower in patients with asymptomatic or mild COVID-19 infections than in those hospitalized in studies analyzing the sera of patients with COVID-19 infections[27,28]. Wu et al.[29] identified no high levels of NAb in 30% of the patients they followed-up and a very low level of NAb in some of their patients, although the duration of illness was similar in those. The production of NAbs has been reported to attain its peak concentration in the first 10-15 days, and then to decrease subsequently after a period of plateauing in COVID-19[28,29]. In the present study, NAb was detected in patients at the earliest on day 14 and at the latest on day 123. The highest NAb presence was recorded in three male patients in 1/80 diluted serum samples. Similar to other studies, the NAb titers in the present study were found to be high in the first 30 days of the disease and were then observed to drop gradually, although an increase in the NAb titers was seen in four patients over time[28-30]. These patients were thought to have been reinfected with the virus during the study period without presenting any symptoms. It is suggested that the plasma collected at four months after PCR positivity can be more useful in the convalescent plasma therapy, since it contains a higher titer of NAbs. Some studies have reported reinfection after several months[31]. This may be the result of the early reduction of NAb titers.

Study Limitations

The present study had some limitations. First, the study was conducted in a single center. Secondly, the fact that individuals with asymptomatic infections could not be detected in the period may have had a small effect on the results, as the distinction between antibodies originating from natural infection and reinfection-elicited antibodies will be affected.

Conclusion

The combined use of kits for the detection of antibodies against different antigens or testing total antibodies would result in a more accurate and earlier detection of the antibodies that start to emerge on the seventh day and decrease after six months of SARS-CoV-2 PCR positivity. In addition, the decrease in neutralizing antibody titers after four months may be one of the causes of early reinfections, although only 12 samples were available from 43 individuals included in the study after the fourth month of the study.

Ethics

Ethics Committee Approval: The study was conducted after being granted approval by the COVID-19 Scientific Research Evaluation Committee of the General Directorate of Health Care Services of the Ministry of Health of the Republic of Turkey, and the Scientific Research Ethics Board of the Medical Faculty of Karadeniz Technical University (protocol no: 20207362, date: February 10, 2021).

Informed Consent: All the patients or their legal guardians signed the informed consent form.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: E.Ö., İ.T., F.A., M.E, Design: E.Ö., İ.T., I.B., C.K.B., N.K., F.A., Data Collection or Processing: E.Ö., İ.T., I.B., C.K.B., F.A., Analysis or Interpretation: E.Ö., İ.T., I.B., C.K.B., N.K., F.A., M.E., Literature Search: E.Ö., İ.T., I.B., C.K.B., N.K., Writing: E.Ö., İ.T., I.B., C.K.B., N.K., F.A.

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References

1. Lei Q, Li Y, Hou HY, Wang F, Ouyang ZQ, Zhang Y, Lai DY, Banga Ndzouboukou JL, Xu ZW, Zhang B, Chen H, Xue JB, Lin XS, Zheng YX, Yao ZJ, Wang XN, Yu CZ, Jiang HW, Zhang HN, Qi H, Guo SJ, Huang SH, Sun ZY, Tao SC, Fan XL. Antibody dynamics to SARS-CoV-2 in asymptomatic COVID-19 infections. Allergy. 2021;76:551-61.
2. Ejazi SA, Ghosh S, Ali N. Antibody detection assays for COVID-19 diagnosis: an early overview. Immunol Cell Biol. 2021;99:21-33.
3. Özçürümez MK, Ambrosch A, Frey O, Haselmann V, Holdenrieder S, Kiehntopf M, Neumaier M, Walter M, Wenzel F, Wölfel R, Renz H; COVID-19 Task Force of the German Society for Clinical Chemistry and Laboratory Medicine (DGKL). SARS-CoV-2 antibody testing-questions to be asked. J Allergy Clin Immunol. 2020;146:35-43.
4. Durmaz B. Microbiological Diagnosis in COVID-19 Infection. Journal of Health Science Yuksek Ihtisas University. 2020;1:12-7.
5. Ali MG, Zhang Z, Gao Q, Pan M, Rowan EG, Zhang J. Recent advances in therapeutic applications of neutralizing antibodies for virus infections: an overview. Immunol Res. 2020;68:325-39.
6. Zhao J, Zhao S, Ou J, Zhang J, Lan W, Guan W, Wu X, Yan Y, Zhao W, Wu J, Chodosh J, Zhang Q. COVID-19: Coronavirus Vaccine Development Updates. Front Immunol. 2020;11:602256.
7. Liu A, Li Y, Peng J, Huang Y, Xu D. Antibody responses against SARS-CoV-2 in COVID-19 patients. J Med Virol. 2021;93:144-8.
8. Amanat F, White KM, Miorin L, Strohmeier S, McMahon M, Meade P, Liu WC, Albrecht RA, Simon V, Martinez-Sobrido L, Moran T, García-Sastre A, Krammer F. An In Vitro Microneutralization Assay for SARS-CoV-2 Serology and Drug Screening. Curr Protoc Microbiol. 2020;58:e108.
9. Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, Li Y, Zhu L, Wang N, Lv Z, Gao H, Ge X, Kan B, Hu Y, Liu J, Cai F, Jiang D, Yin Y, Qin C, Li J, Gong X, Lou X, Shi W, Wu D, Zhang H, Zhu L, Deng W, Li Y, Lu J, Li C, Wang X, Yin W, Zhang Y, Qin C. Development of an inactivated vaccine candidate for SARS-CoV-2. Science. 2020;369:77-81.
10. Wang B, Wang L, Kong X, Geng J, Xiao D, Ma C, Jiang XM, Wang PH. Long-term coexistence of SARS-CoV-2 with antibody response in COVID-19 patients. J Med Virol. 2020;92:1684-9.
11. Liu A, Li Y, Peng J, Huang Y, Xu D. Antibody responses against SARS-CoV-2 in COVID-19 patients. J Med Virol. 2021;93:144-8.
12. Younes N, Al-Sadeq DW, Al-Jighefee H, Younes S, Al-Jamal O, Daas HI, Yassine HM, Nasrallah GK. Challenges in Laboratory Diagnosis of the Novel Coronavirus SARS-CoV-2. Viruses. 2020;12:582.
13. Jääskeläinen AJ, Kuivanen S, Kekäläinen E, Ahava MJ, Loginov R, Kallio-Kokko H, Vapalahti O, Jarva H, Kurkela S, Lappalainen M. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. J Clin Virol. 2020;129:104512.
14. Gao YD, Ding M, Dong X, Zhang JJ, Kursat Azkur A, Azkur D, Gan H, Sun YL, Fu W, Li W, Liang HL, Cao YY, Yan Q, Cao C, Gao HY, Brüggen MC, van de Veen W, Sokolowska M, Akdis M, Akdis CA. Risk factors for severe and critically ill COVID-19 patients: A review. Allergy. 2021;76:428-55.
15. Lee YL, Liao CH, Liu PY, Cheng CY, Chung MY, Liu CE, Chang SY, Hsueh PR. Dynamics of anti-SARS-Cov-2 IgM and IgG antibodies among COVID-19 patients. J Infect. 2020;81:e55-8.
16. Wang B, Wang L, Kong X, Geng J, Xiao D, Ma C, Jiang XM, Wang PH. Long-term coexistence of SARS-CoV-2 with antibody response in COVID-19 patients. J Med Virol. 2020;92:1684-9.
17. Laing ED, Epsi NJ, Richard SA, Samuels EC, Wang W, Vassell R, Ewing DF, Herrup R, Sterling SL, Lindholm DA, Millar EV, Maves RC, Larson DT, Colombo RE, Chi S, Madar C, Lalani T, Ganesan A, Fries A, Colombo CJ, Mende K, Simons MP, Schully KL, Weiss CD, Burgess TH, for the EPICC Study team. SARS-CoV-2 antibodies remain detectable 12 months after infection and antibody 2 magnitude is associated with age and COVID-19 severity. medRxiv. doi:10.1101/2021.04.27.21256207.
18. Crawford KHD, Dingens AS, Eguia R, Wolf CR, Wilcox N, Logue JK, Shuey K, Casto AM, Fiala B, Wrenn S, Pettie D, King NP, Greninger AL, Chu HY, Bloom JD. Dynamics of Neutralizing Antibody Titers in the Months After Severe Acute Respiratory Syndrome Coronavirus 2 Infection. J Infect Dis. 2021;3;223:197-205.
19. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. Epidemiol Infect. 1990;105:435-46.
20. Kellam P, Barclay W. The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection. J Gen Virol. 2020;101:791-7.
21. Ong DSY, Fragkou PC, Schweitzer VA, Chemaly RF, Moschopoulos CD, Skevaki C; European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Respiratory Viruses (ESGREV). How to interpret and use COVID-19 serology and immunology tests. Clin Microbiol Infect. Clin Microbiol Infect. 2021;27:981-6.
22. Føns S, Krogfelt KA. How can we interpret SARS-CoV-2 antibody test results? Pathog Dis. 2021;79:ftaa069.
23. Van Elslande J, Decru B, Jonckheere S, Van Wijngaerden E, Houben E, Vandecandelaere P, Indevuyst C, Depypere M, Desmet S, André E, Van Ranst M, Lagrou K, Vermeersch P. Antibody response against SARS-CoV-2 spike protein and nucleoprotein evaluated by four automated immunoassays and three ELISAs. Clin Microbiol Infect. 2020;26:1557.e1-7.
24. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, Yip CC, Cai JP, Chan JM, Chik TS, Lau DP, Choi CY, Chen LL, Chan WM, Chan KH, Ip JD, Ng AC, Poon RW, Luo CT, Cheng VC, Chan JF, Hung IF, Chen Z, Chen H, Yuen KY. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020;20:565-74.
25. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, Hu JL, Xu W, Zhang Y, Lv FJ, Su K, Zhang F, Gong J, Wu B, Liu XM, Li JJ, Qiu JF, Chen J, Huang AL. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020;26:1200-4.
26. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, Grifoni A, Ramirez SI, Haupt S, Frazier A, Nakao C, Rayaprolu V, Rawlings SA, Peters B, Krammer F, Simon V, Saphire EO, Smith DM, Weiskopf D, Sette A, Crotty S. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science. 2021;371:eabf4063.
27. Grzelak L, Temmam S, Planchais C, Demeret C, Tondeur L, Huon C, Guivel-Benhassine F, Staropoli I, Chazal M, Dufloo J, Planas D, Buchrieser J, Rajah MM, Robinot R, Porrot F, Albert M, Chen KY, Crescenzo-Chaigne B, Donati F, Anna F, Souque P, Gransagne M, Bellalou J, Nowakowski M, Backovic M, Bouadma L, Le Fevre L, Le Hingrat Q, Descamps D, Pourbaix A, Laouénan C, Ghosn J, Yazdanpanah Y, Besombes C, Jolly N, Pellerin-Fernandes S, Cheny O, Ungeheuer MN, Mellon G, Morel P, Rolland S, Rey FA, Behillil S, Enouf V, Lemaitre A, Créach MA, Petres S, Escriou N, Charneau P, Fontanet A, Hoen B, Bruel T, Eloit M, Mouquet H, Schwartz O, van der Werf S. A comparison of four serological assays for detecting anti-SARS-CoV-2 antibodies in human serum samples from different populations. Sci Transl Med. 2020;12:eabc3103.
28. Brochot E, Demey B, Touzé A, Belouzard S, Dubuisson J, Schmit JL, Duverlie G, Francois C, Castelain S, Helle F. Anti-spike, Anti-nucleocapsid and Neutralizing Antibodies in SARS-CoV-2 Inpatients and Asymptomatic Individuals. Front Microbiol. 2020;11:584251.
29. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, Ling Y, Zhang Y, Xun J, Lu L, Jiang S, Lu H, Wen Y, Huang J. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv. https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2.

30. Batra M, Tian R, Zhang C, Clarence E, Sacher CS, Miranda JN, De La Fuente JRO, Mathew M, Green D, Patel S, Bastidas MVP, Haddadi S, Murthi M, Gonzalez MS, Kambali S, Santos KHM, Asif H, Modarresi F, Faghihi M, Mirsaeidi M. Role of IgG against N-protein of SARS-CoV2 in COVID19 clinical outcomes. Sci Rep. 2021;11:3455.

31. Van Elslande J, Vermeersch P, Vandervoort K, Wawina-Bokalanga T, Vanmechelen B, Wollants E, Laenen L, André E, Van Ranst M, Lagrou K, Maes P. Symptomatic Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Reinfection by a Phylogenetically Distinct Strain. Clin Infect Dis. 2021;73:354-6.