Detection of anti-SARS-CoV-2 nucleocapsid and spike antibodies in patients with Coronavirus Disease 2019 in Japan.

Hiroshi Furukawa (furukawa-tky@umin.org)
National Hospital Organization Tokyo National Hospital
Shomi Oka
National Hospital Organization Tokyo National Hospital
Takashi Higuchi
National Hospital Organization Tokyo National Hospital
Miho Yamaguchi
National Hospital Organization Tokyo National Hospital
Shota Uchiyama
National Hospital Organization Tokyo National Hospital
Tomohiro Koiwa
National Hospital Organization Tokyo National Hospital
Moriyuki Nakama
National Hospital Organization Tokyo National Hospital
Masaaki Minegishi
National Hospital Organization Tokyo National Hospital
Hideaki Nagai
National Hospital Organization Tokyo National Hospital
Shigeto Tohma
National Hospital Organization Tokyo National Hospital

Research Article

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Abstract

Objectives Coronavirus Disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Serological testing for anti-SARS-CoV-2 nucleocapsid (N) antibodies (Abs) and anti-SARS-CoV-2 spike (S) Abs is performed to detect prior COVID-19 infection. It is still controversial which antibodies are the most sensitive and specific, and which can be detected earliest after infection. Here, we evaluated the results of serological tests of anti-SARS-CoV-2 N and S Abs in Japan.

Methods Symptomatic COVID-19 patients (n = 84) and control patients with rheumatoid arthritis (n = 93) were recruited at Tokyo National Hospital. Anti-SARS-CoV-2 N and S Abs were measured by commercial electrochemiluminescence immunoassays.

Results The fraction of patients positive for anti-SARS-CoV-2 N and S Abs was highest > 14 days after symptom onset. The frequency of anti-SARS-CoV-2 S Ab positivity at this time (80.4%) tended to be slightly but not significantly lower than anti-SARS-CoV-2 N Ab positivity (84.8%). Optimized cut-off levels for anti-SARS-CoV-2 N and S Ab positivity were lower than the manufacturer's recommended cut-off levels. Using multiple linear regression analyses with anti-SARS-CoV-2 N and S Abs, we created an Ab-index with high sensitivity.

Conclusion To increase the sensitivity of serological diagnostic tests for COVID-19, it is suggested that both anti-SARS-CoV-2 N and S Abs should be measured and cut-off levels decreased.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19) which emerged in December 2019 in Wuhan, China [1]. Real-time reverse transcription polymerase chain reaction detection of SARS-CoV-2 in nasopharyngeal swabs, saliva, sputum or bronchoalveolar lavage samples is the standard method of COVID-19 virological diagnosis [2]. Serological detection of anti-SARS-CoV-2 antibodies (Abs) is necessary for the detection of prior infection or evaluation of vaccine efficacy. The major structural proteins of SARS-CoV-2 include spike (S), nucleocapsid (N), envelope, and membrane proteins [3]. The S and N proteins possess immunogenic epitopes and are potential target antigens for serological assays [4–11] which have accordingly been developed against both. It is unknown how long these antibodies persist after COVID-19 infection and is controversial which antibodies are the most sensitive, specific, or first to be detected after infection. Hence, we evaluated the results of serological tests for anti-SARS-CoV-2 N and S Abs in Japan using commercial electrochemiluminescence immunoassays (ECLIA).

Materials And Methods

Patients and sera

Symptomatic COVID-19 patients recruited at Tokyo National Hospital from April 2020 to February 2021 (n = 84) were diagnosed by real-time reverse transcription polymerase chain reaction or loop-mediated isothermal amplification methods. [12] Severity of COVID-19 was defined as follows: patients not requiring oxygen (severity 1), patients requiring oxygen (severity 2), and patients requiring mechanical ventilation or deceased due to respiratory failure (severity 3). Control patients with rheumatoid arthritis were also recruited at Tokyo National Hospital (n = 93), not diagnosed as having COVID-19 before serum collection. Sera from these two groups of patients were analyzed for anti-SARS-CoV-2 Abs. This study was approved by The Research Ethics Committee of Tokyo National Hospital, which has waived written informed consent for emerging infectious diseases. Oral informed consent was obtained from the patients with COVID-19 and written informed consent was obtained from the control patients. This study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

Anti-SARS-CoV-2 Ab analyses

IgM and IgG classes of anti-SARS-CoV-2 N Abs were detected using the ECLIA system (Elecsys Anti-SARS-CoV-2, Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions, and cut-off indices (COI) were calculated. Samples with COI < 1.0 were considered negative for anti-SARS-CoV-2 N Abs; samples with COI ≥ 1.0 were considered positive. Results of anti-SARS-CoV-2 N Ab assays for the control patients with rheumatoid arthritis have already been reported [13]. IgM and IgG classes of anti-SARS-CoV-2 S Abs were also detected using the ECLIA system (Elecsys Anti-SARS-CoV-2 S, Roche Diagnostics), according to the manufacturer's instructions, and COI were calculated. Samples with COI < 0.8 were negative and those with COI ≥ 0.8 were positive.

Statistical analysis

Differences between characteristics of the patients with COVID-19 or control patients were analyzed by Student's t-test or Fisher's exact test using 2x2 contingency tables. The area under the curve (AUC) values of the receiver operating characteristic (ROC) curves for anti-SARS-CoV-2 N Abs and for anti-SARS-CoV-2 S Abs were compared with the AUC value of 0.5 by Chi-square analysis. From ROC curves, optimized cut-off levels, sensitivities, and specificities were calculated based on the highest Youden's index. Multiple linear regression analyses of anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs were performed to create Ab-indices as new biomarkers.

Results

Characteristics of the patients

The characteristics of the patients are shown in Table 1. The mean age of the patients with COVID-19 was lower than controls and the proportion of men was higher.

Table 1
Characteristics of COVID-19 patients and controls.

	COVID-19 patients	Controls	*P*
Number	84	93	
Mean age, years (SD)	58.8 (19.5)	73.4 (10.7)	$3.96X10^{-8}$
Male, n (%)	56 (66.7)	23 (24.7)	$*3.13X10^{-8}$
Severity 2 or 3, n (%)	24 (28.6)		

Numbers or average values are shown. Standard deviations or percentages are shown in parentheses. Significance of the differences were tested by Fisher's exact test using 2x2 contingency tables or Student's *t*-test. *Fisher's exact test was employed. COVID-19: coronavirus disease 2019, SD: standard deviation.

Anti-SARS-CoV-2 antibodies in sera from COVID-19 patients

Anti-SARS-CoV-2 N Abs were assessed in patients with COVID-19 (Table 2). The frequency of patients positive for anti-SARS-CoV-2 N Abs was low 0–6 days after symptom onset (3.7%), but reached 84.8% >14 days later. Anti-SARS-CoV-2 S Abs were also measured in patients with COVID-19. The frequency was also low 0–6 days after symptom onset (5.6%), rising to 66.7% 7–13 days thereafter. This was slightly higher than the frequency of anti-SARS-CoV-2 N Abs (56.5%), although this difference did not achieve statistical significance ($P = 0.2936$). The frequency of patients with anti-SARS-CoV-2 S Abs after day 14 (80.4%) tended to be lower than for anti-SARS-CoV-2 N Abs (84.8%), but this was again not significant ($P = 0.7841$). The frequency of COVID-19 patients with one or the other anti-N or anti-S Abs was highest after day 14 (93.5%), suggesting that measurement of both Abs should be used for diagnosis of COVID-19. The frequency of anti-SARS-CoV-2 N and S Abs in COVID-19 patients stratified by age, sex, or disease severity is shown in Supplementary Table S1, indicating no influence of these variables. Thus, the frequency of all patients positive for anti-SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs was highest after day 14. In contrast, no SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs were detected in control patients with rheumatoid arthritis.

Table 2
Frequencies of anti-SARS-CoV-2 N and S Abs in serum samples of COVID-19 patients after symptom onset.

	Days since symptom onset		
	Day 0–6	Day 7–13	Day 14-
Anti-SARS-CoV-2 N Ab-positive	2 (3.7)	39 (56.5)	39 (84.8)
Anti-SARS-CoV-2 S Ab-positive	3 (5.6)	46 (66.7)	37 (80.4)
Both anti-SARS-CoV-2 N and S Ab-positive	1 (1.9)	34 (49.3)	33 (71.7)
Anti-SARS-CoV-2 N or S Ab-positive	4 (7.4)	51 (73.9)	43 (93.5)

Number of positive patients in each group is shown, with percentages in parentheses. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SARS-CoV-2 N: SARS-CoV-2 nucleocapsid protein, SARS-CoV-2 S: SARS-CoV-2 spike protein, Ab: antibody.

The AUC values of the ROC curves for anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs are shown in Fig. 1. The AUC values on day 0–6 after symptom onset were lowest, and those after day 14 were highest (anti-SARS-CoV-2 N Abs: AUC, 0.954; 95% confidence intervals [CI] 0.911–0.997, $P = 3.97X10^{-95}$, and for anti-SARS-CoV-2 S Abs: AUC, 0.913; 95% CI 0.858–0.968, $P = 2.09X10^{-48}$). From these ROC curves, optimized cut-off levels, sensitivities, and specificities were calculated based on the highest Youden's index. The optimized cut-off level of anti-SARS-CoV-2 N Abs was 0.20; this is lower than the manufacturer´s recommended cut-off level of 1.0. The optimized cut-off level of anti-SARS-CoV-2 S Abs was 0.44, also lower than the recommended cut-off level of 0.8. These data suggest that lower cut-off values should be used for these Abs for a better sensitivity.

Multiple linear regression analyses of anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs > 14 days after symptom onset were performed and an Ab-index was generated as (COI of anti-SARS-CoV-2 N Abs) X 0.0120 + (COI of anti-SARS-CoV-2 S Abs) X 0.0003 + 0.2325. A ROC curve was calculated with an AUC of 0.965 (95% CI 0.927-1.000, $P = 2.61X10^{-129}$, Fig. 2), which is higher than for anti-SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs alone. Sensitivities and specificities were calculated and the sensitivity with the highest Youden's index (0.935) was found to be higher than for either anti-SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs. Thus, multiple linear regression analyses with anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs resulted in the creation of an Ab-index with high sensitivity.

Discussion

In the present study, the diagnostic performance of anti-SARS-CoV-2 N Ab and anti-SARS-CoV-2 S Ab according to their detection by Elecsys was investigated. It was found that the frequency of COVID-19 patients with anti-SARS-CoV-2 S Abs during the 7–13 days after symptom onset was slightly higher than for anti-SARS-CoV-2 N Abs. However, after day 14, the frequency of anti-SARS-CoV-2 S Abs was lower than that of anti-SARS-CoV-2 N Abs (Table 2). These data suggest earlier production, higher specificity,

and lower sensitivity of anti-SARS-CoV-2 S relative to N Abs. However, the sensitivity of anti-SARS-CoV-2 S Abs was higher, although the levels of these Abs were increased earlier in a previous study [14]. It was reported that anti-SARS-CoV-2 S Abs were more specific due to the lower cross-reactivity with S proteins of other coronaviruses [15–18], supporting the results obtained in our study (Fig. 1).

The frequency of patients with anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs was not sufficiently high and the rate of “anti-SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs” positivity after day 14 was higher than for either of these Abs alone (Table 2). The sensitivities of these Abs were relatively lower after day 14 (Fig. 1). However, the sensitivity of the Ab-index was higher than that of anti-SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs separately (Fig. 2). These data suggest that both anti-SARS-CoV-2 N and S Abs should be measured in order to increase the sensitivity of serological diagnostic methods for COVID-19. On the other hand, specificities of anti-SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs are high enough and the false positive rates of these Abs low. In the ROC curve analyses in our study, the optimized cut-off levels of anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs were lower compared than the manufacturer's recommended cut-off levels (Fig. 1). We therefore suggest to decrease the cut-off levels of anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs for improvement of the serological diagnostic tests of COVID-19.

It was reported that serum IgM levels of anti-SARS-CoV-2 N and S Abs decreased after day 18, though IgG levels were maintained [19, 20]. In the method used for detection in the present study, serum IgM and IgG levels were measured for anti-SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs. Therefore, these characteristics of IgM and IgG separately could not be evaluated here. Because the sample size of this study is modest, further large-scale studies are necessary to validate the results presented here. Antibody profiles in asymptomatic patients with COVID-19 or controls other than rheumatoid arthritis patients were not analyzed in this study; these are the limitations of the present study. Antibody profiles should be measured in future studies with this in mind.

Conclusion

This study characterized the diagnostic performance of anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs in patients with COVID-19 in Japan. To increase the sensitivity of serological diagnostic tests for COVID-19, it is suggested to measure both anti-SARS-CoV-2 N Abs or anti-SARS-CoV-2 S Abs and to decrease the cut-off levels for assigning positivity for these Abs. These amendments could extend the application of serological diagnostic tests for COVID-19.

Abbreviations

CI:confidence interval, SARS-CoV-2:severe acute respiratory syndrome coronavirus 2, N:nucleocapsid, S:spike, COVID-19:coronavirus disease 2019, SD:standard deviation, ECLIA:electrochemiluminescence immunoassay, Ab:antibody, COI:cut-off index, AUC:area under the curve, ROC:receiver operating characteristic.

Declarations

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Author Contributions

Conceived and designed the experiments: HF and ST, Performed the experiments: SO, TH, and HF, Analyzed the data: HF and ST, Contributed reagents/materials/analysis tools: HF, SO, TH, MY, SU, TK, HN, and ST. Wrote the manuscript: HF and ST.

Conflict of interest statement

HF was supported by research grants from Bristol-Myers Squibb Co. ST was supported by research grants from 9 pharmaceutical companies: Abbott Japan Co., Ltd., Astellas Pharma Inc., Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Merck Sharp and Dohme Inc., Pfizer Japan Inc., Takeda Pharmaceutical Company Limited, Teijin Pharma Limited. The other authors declare no financial or commercial conflict of interest.

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Figures

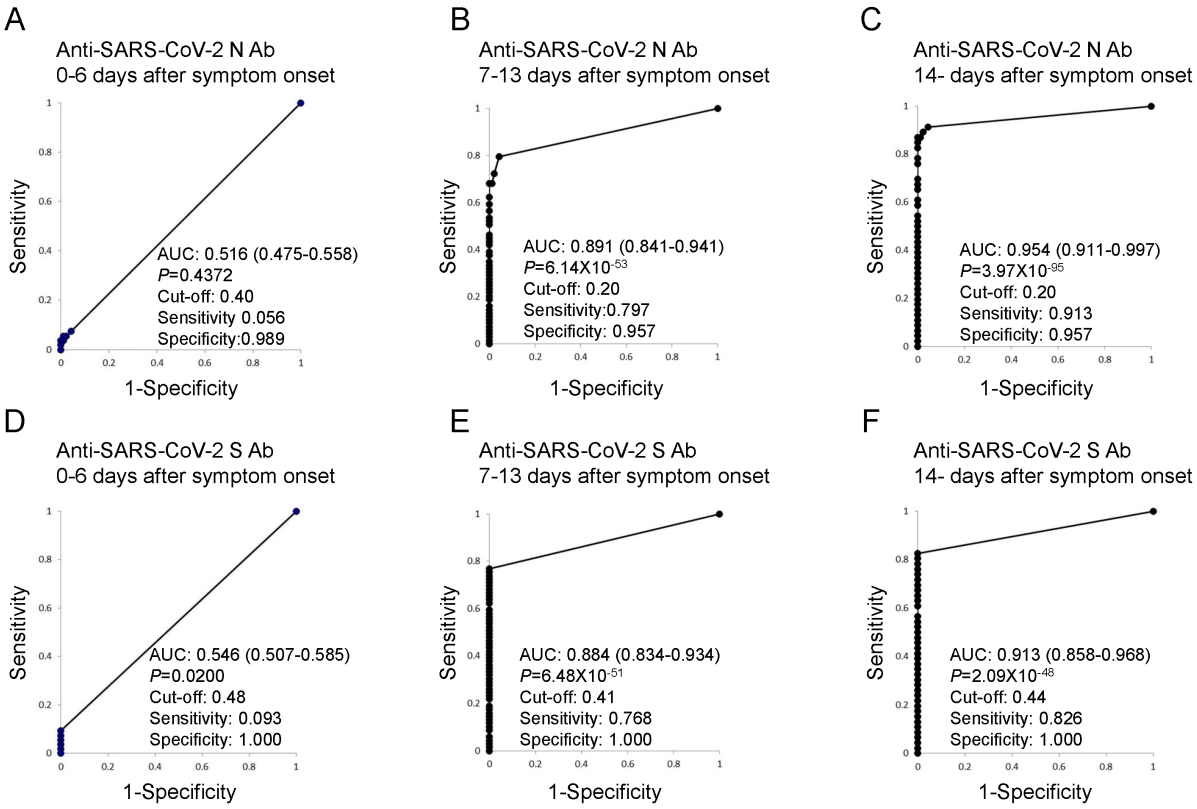

Figure 1

Receiver operating characteristic (ROC) curves using anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs. ROC curves for anti-SARS-CoV-2 N Ab 0-7 (A), 8-13 (B), and >14 (C) days after symptom onset. ROC curves for anti-SARS-CoV-2 S Ab 0-7 (D), 8-13 (E), and >14 (F) days after symptom onset. The area under the curve (AUC) values of the ROC curves with 95% confidence intervals and the optimized cut-off levels with specificities and sensitivities are depicted. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SARS-CoV-2 N: SARS-CoV-2 nucleocapsid protein, SARS-CoV-2 S: SARS-CoV-2 spike protein, ROC: receiver operating characteristic, AUC: area under the curve.

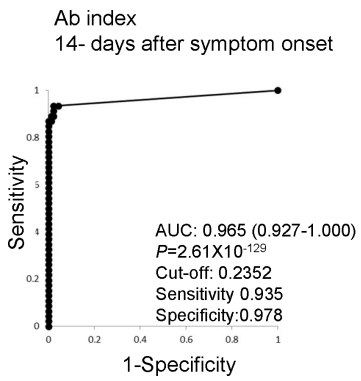

Figure 2

Receiver operating characteristic (ROC) curves using multiple regression analysis with the anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs. Multiple linear regression analyses of anti-SARS-CoV-2 N Abs and anti-SARS-CoV-2 S Abs >14 days after symptom onset were performed and an Ab-index was generated to create a complex biomarker for COVID-19. The ROC curve of the Ab-index, the area under the curve (AUC) value of the ROC curve with 95% confidence intervals, and the optimized cut-off level with the specificity and sensitivity are depicted. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SARS-CoV-2 N: SARS-CoV-2 nucleocapsid protein, SARS-CoV-2 S: SARS-CoV-2 spike protein, ROC: receiver operating characteristic, AUC: area under the curve.

Supplementary Files

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- TableS1.pdf