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## Original Article

## Survey of the current status of subclinical coronavirus disease 2019 (COVID-19)



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## ABSTRACT

**Objectives:** We investigated relationships between subclinical COVID-19 (coronavirus disease 2019) and background factors.

**Methods:** We determined SARS-CoV-2 antibody (IgG) prevalence in 1603 patients, doctors, and nurses in 65 medical institutions in Kanagawa Prefecture, Japan and investigated their background factors. Antibodies (IgG) against SARS-CoV-2 were analyzed by Immunochromatographic test.

**Results:** The 39 subjects (2.4%) were found to be IgG antibody-positive: 29 in the patient group (2.9%), 10 in the doctor/nurse group (2.0%), and 0 in the control group. After adjustment for age, sex, and the antibody prevalence in the control group, antibody prevalence was 2.7% in the patient group and 2.1% in the doctor/nurse group. There was no significant difference between the antibody-positive subjects and the antibody-negative subjects in any background factors investigated including overseas travel, contact with overseas travelers, presence/absence of infected individuals in the living area, use of trains 5 times a week or more, BCG vaccination, and use of ACE inhibitor and ARB.

**Conclusions:** Antibody prevalence in the present survey at medical institution is higher than that in Tokyo and in Osaka measured by the government suggesting that subclinical infections are occurring more frequently than expected. No background factor that influenced antibody-positive status due to subclinical infection was identified.

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## 1. Introduction

The COVID-19 (coronavirus disease 2019) pandemic caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has spread worldwide [1]. Although serious COVID-19 can be a fatal disease, asymptomatic or mild cases of SARS-CoV-2 infections have been found [2–5]. It has also been reported that individuals with asymptomatic COVID-19 and those before developing symptoms can be infectious [6,7], suggesting that those with subclinical infection can contribute to the spread of infection.

In reports from Japan, the antibody prevalence ranged from 0.03% to 3.3% [8–11]. The reported range in survey results of other countries was wider, from approximately 1% to over 10% [12–16].

In previous reports, however, relationships between background information such as behavior and concomitant diseases of subjects and antibody prevalence are not clear [2,17]. There was also a report suggesting that BCG vaccination protects vaccinees against viral infections [18].

## 2. Material and methods

## 2.1. Study design

This is a multicenter epidemiological study in 65 medical institutions in Kanagawa Prefecture. Patients were enrolled from May 18 to June 24, 2020.

The subjects in this study were those who met all inclusion criteria below and did not violate any exclusion criterion. (rationale for each criterion is shown in parentheses).

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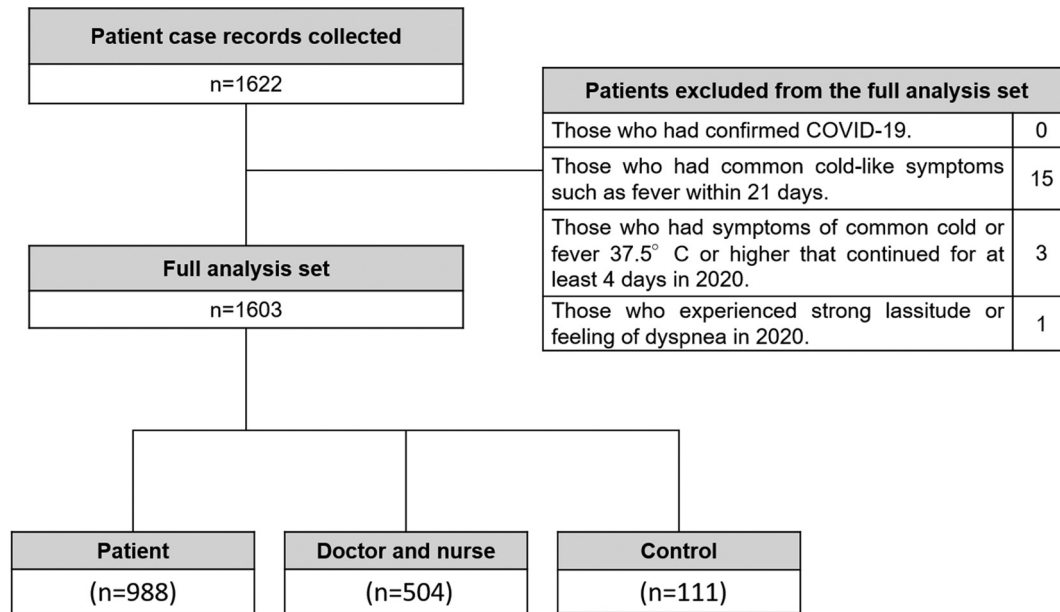


Fig. 1. Participant flow.

## 2.2. Inclusion criteria

- 1) Patients who regularly visit medical institutions that belong to the Kanagawa Physicians Association, or doctors or nurses who work in medical institutions that belong to the Kanagawa Physicians Association (because this is a study in Kanagawa Prefecture and in order to know the current status of subclinical infections among doctors and nurses)
- 2) Any age (to collect data from a wide range of age groups)
- 3) Those who live in Japan and can be followed up continuously (positive patients may be followed up continuously)
- 4) Those who gave consent to participate in the study (including consent from legal guardians for minors)

## 2.3. Exclusion criteria

- 1) Those who had confirmed COVID-19 (because this study investigates the current status of subclinical infections)
- 2) Those who had common cold-like symptoms such as fever within 21 days (to prevent transmission to healthcare workers. It has been reported that IgG antibodies against SARS-CoV-2 increase 2–3 weeks after the onset of COVID-19 [19])
- 3) Those who had symptoms of common cold or fever 37.5 °C or higher that continued for at least 4 days in 2020 (these individuals may have been infected with the novel coronavirus and are not suitable for the investigation of subclinical infection)
- 4) Those who experienced strong lassitude or feeling of dyspnea in 2020 (the same as above)

\*When this study was started, the government was ordering that those who have symptoms described in exclusion criteria 3) and 4) should go to specialized medical institutions because infection with the novel coronavirus is suspected.

## 2.4. Method

The study was explained to subjects using written documents, and their consent was obtained in writing. Their answers to a

questionnaire were collected. The blood was drawn, and antibody testing was performed. If the result of antibody testing was positive, the subject was contacted immediately after the result was obtained, and if necessary, a Polymerase Chain Reaction (PCR) test was performed.

## 2.5. Assay kit

Cica Immuno-test SARS-CoV-2 IgG was used [20]. This is a reagent developed through collaborative research by Professor Akihide Ryo of the Department of Microbiology, Yokohama City University Graduate School of Medicine and Kanto Chemical Co., Inc., which detects human anti-SARS-CoV-2 antibodies (IgG) contained in the serum of individuals infected with the novel coronavirus (supplementary material 1). The sensitivity of the assay kit was as follows: 89% (8/9) for 9 positive-control specimens (9–12 days after onset) and 100% (8/8) for 8 positive-control specimens (13 days after onset or later). Regarding specificity/cross reactivity, all negative controls (20 specimens of human derived sera before the epidemic) were negative. In terms of reproducibility, all positive controls (triplicate measurements) were positive and all negative controls (triplicate measurements) were negative. The positive controls were sera from PCR-positive patients and the negative controls were sera collected before the endemic.

## 2.6. Survey items

Age; sex; body height; body weight; smoking habit; drinking habit; presence/absence of underlying disease (hypertension, dyslipidemia, diabetes, hyperuricemia, cerebrovascular disease, heart disease, thromboembolism, obesity, lung disease, liver disease, kidney disease, and immunological disease); use of angiotensin-receptor blocker (ARB) or angiotensin-converting-enzyme inhibitor (ACEI); history of BCG vaccination; overseas travel in 2020; presence/absence of symptoms of common cold in 2020; presence/absence of symptoms that can be due to COVID-19; presence/absence of individuals infected with SARS-CoV-2 among family members, in the workplace, school, and other places; history of influenza in 2020, contact with overseas travelers or travelers who

visited Japan in 2020; use of air purifiers; average sleep time; and use of trains 5 times a week or more. (for doctors and nurses, occupation, specialty, hospital/clinic, and outpatient/general ward/ICU were additionally surveyed).

## 2.7. Endpoints

The primary endpoint was the proportion of SARS-CoV-2 antibody (IgG)-positive subjects in the entire study population. Of all participants, those who were not doctors or nurses and who had no underlying disease were categorized into the control group, and antibody prevalence in the control group, the patient group (with underlying disease), and the doctor/nurse group was calculated. Moreover, antibody prevalence in the patient group and the doctor/nurse group adjusted for age, sex, and the antibody prevalence in the control group was calculated. The SARS-CoV-2 antibody (IgG)-positive group and the negative group were compared in terms of each survey item. Moreover, factors for antibody-positive status were analyzed. \*control group: The subjects in this group have undergone medical examinations or vaccinations.

## 2.8. Statistical analyses

The data were analyzed using R version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria [<https://www.R-project.org/>]). When comparing patient background, the Fisher's exact test was used for nominal variables and ANOVA or the Student's t-test was used for continuous variables. If the answer to a questionnaire survey item was unknown, the datum corresponding to it was excluded from statistical test. The significance level was 5% on either side. Demographic characteristics are presented as the mean  $\pm$  standard deviation or the number of cases (%).

When antibody prevalence in the patient group and the doctor/nurse group adjusted for age, sex, and the antibody prevalence in the control group was calculated, the analytical procedure was [supplementary material 2](#).

Factors for antibody-positive status were also analyzed exploratively using the multiple logistic regression analysis. The response variable was antibody test results. Explanatory variables were age, sex, BMI, current smoker, current drinker, average sleep time, BCG vaccination, contact with overseas travelers or travelers who visited Japan in 2020, presence/absence of individuals infected with the novel coronavirus in the living area, use of air purifiers at home, use of trains 5 times a week or more, lung disease, and subject group (doctor/nurse or not), which were analyzed as combinations.

## 3. Results

There were 1603 subjects who met the inclusion criteria and did not violate any exclusion criterion: 988 patients, 217 doctors, 287 nurses, and 111 control subjects (no underlying disease) ([Fig. 1](#), [Supplementary material 3](#)). The number of subjects positive for anti-novel coronavirus antibodies were 39 in the entire study population (2.4%), 29 in the patient group (2.9%), 10 in the doctor/nurse group (2.0%), and 0 in the control group. After adjustment for age, sex, and the antibody prevalence in the control group, antibody prevalence was 2.7% in the patient group and 2.1% in the doctor/nurse group ([Supplementary material 4](#)).

There was no significant difference between the antibody-positive subjects and the antibody-negative subjects in subject background ([Table 1](#)). Multiple logistic regression analyses were performed with multiple patterns to investigate factors for antibody-positive status. However, none of the combinations tested led to the identification of factors that influenced antibody-positive status.

Although there were 85 subjects who traveled abroad including travel to where the novel coronavirus epidemic occurred, their antibody test results were all negative.

Subjects who were found to be antibody-positive underwent a PCR test if their physicians judged it necessary. 7 out of 39 antibody-positive individuals underwent PCR testing. However, all of them were PCR-negative. Even if the antibody test was positive, the possibility of current infection/onset could not be ruled out. So, in some cases, PCR tests were performed. In some cases, the tests were refused by public health centers/medical associations. Some patients did not receive a PCR test because he did not have any symptoms and did not want to get the check.

## 4. Discussion

The present study found that the proportion of subclinical infections was 2.4% in Kanagawa Prefecture.

A survey of 7950 subjects by the Ministry of Health, Labour and Welfare of Japan revealed that anti-novel coronavirus antibody prevalence was 0.10% in Tokyo, 0.17% in Osaka, and 0.03% in Miyagi [9]. According to a survey by SoftBank Group, antibody prevalence was 1.79% among 5850 healthcare workers and 0.23% among 38,216 non-healthcare workers [10]. It was 1.0% in a survey using sera from 312 subjects in Osaka [11]. Antibody prevalence in the present survey is higher than these results. It is the second highest after 3.3% in a survey of serum samples from 1000 residents in Kobe City [8]. In this survey, the following subjects were excluded; those who had symptoms of the common cold or fever 37.5 °C or higher that continued for at least four days in 2020, or those who experienced strong lassitude or feeling of dyspnea in 2020. The actual subclinical infection rate seems to be higher. That's because some people are not aware of SARS-CoV-2 infection, although they have the symptoms listed above.

There are 3 factors described below that may have contributed to the high antibody prevalence in the present study.

First, specimens used were different: whole blood vs. serum. In the present study, the serum was used. Because the whole blood sample contains a less amount of serum component that is involved in antigen-antibody reaction, antibodies can conceivably be detected more accurately when the serum is used. Second, there are many kits the specificity and sensitivity of which have not been clarified. The Japanese Association for Infectious Diseases evaluated the performance of 4 foreign-made assay kits using the plasma/whole blood of 10 patients and reported the results: the sensitivity of the assay kits from 4 companies was 2/5, 0/5, 3/5, and 4/5 [21]. The sensitivity and other characteristics of the kit used in the present survey are clear, as described in the method section. Third, antigens used are different. Grifoni et al. and Thiel et al. reported that cross-reactivity in subjects who were not infected with SARS-CoV-2 [22,23]. Developing an assay in which cross-reactivity is suppressed is an issue. Besides SARS-CoV-2, there are 6 coronaviruses that are known to infect humans (OC43, 229E, SARS-CoV, NL63, HKU1, and MERS-CoV) [24–28]. Antibodies can be cross-reactive to any combination of these human coronaviruses (hCoV). Main viral antigens most frequently used in antibody tests for coronavirus infection are nucleocapsid protein (N) and spike protein (S), 2 of the 4 main structural proteins [29]. Chia et al. investigated the performance of N, S1, and receptor-binding domain (RBD) proteins from SARS-CoV-2 and SARS-CoV using 4 different test platforms and reported that although RBD protein showed the highest specificity, N proteins of both viruses had very high cross-reactivity and are unsuitable for the detection of virus-specific antibodies [30]. An N-terminal region that is highly homologous to other viruses was deleted from the N protein used as the antigen in the immunochromatographic kit used in the present

**Table 1**  
Participant characteristics (positive/negative).

		positive (n = 39)	negative (n = 1564)	p
Sex	Male	16 (41.0%)	694 (44.4%)	0.746
	Female	23 (59.0%)	870 (55.6%)	
Age (y)		59.1 ± 18.9	54.2 ± 15.8	0.061
Body height (cm)		161.1 ± 8.8	162.6 ± 9.3	0.331
Body weight (kg)		61.2 ± 10.6	61.5 ± 13.3	0.876
Body Mass Index (kg/m <sup>2</sup> )		23.5 ± 3.3	23.3 ± 7.5	0.869
Sleep duration (h)		6.6 ± 1.0	6.3 ± 1.1	0.127
Smoking habit	Smoker	1 (2.6%)	186 (11.9%)	0.186
	Previous smoker	9 (23.1%)	339 (21.7%)	
	Non-smoker	29 (74.4%)	1036 (66.2%)	
	Drinker	16 (41.0%)	730 (46.7%)	
Drinking habit	Previous drinker	3 (7.7%)	89 (5.7%)	0.593
	Non-drinker	20 (51.3%)	742 (47.4%)	
	BCG vaccination	35 (89.7%)	1424 (91.0%)	
Overseas travel in 2020		0 (0%)	85 (5.4%)	0.265
Contact with overseas travelers or travelers who visited Japan in 2020		3 (7.7%)	263 (16.8%)	0.186
Individuals infected with the novel coronavirus in the living environments such as home, workplace, school, and other places		1 (2.6%)	101 (6.5%)	0.511
History of influenza in 2020		0 (0%)	34 (2.2%)	1.000
Use of air purifiers at home		11 (28.2%)	634 (40.5%)	0.181
Use of trains 5 times a week or more		8 (20.5%)	326 (20.8%)	1.000
Development of symptoms listed below in 2020				
Cough		1 (2.6%)	99 (6.3%)	0.510
Runny nose		4 (10.3%)	135 (8.6%)	0.770
Sputum		2 (5.1%)	45 (2.9%)	0.318
Headache		1 (2.6%)	66 (4.2%)	1.000
Fever		0 (0%)	78 (5.0%)	0.257
Dysosmia		1 (2.6%)	4 (0.3%)	0.116
Dysgeusia		1 (2.6%)	6 (0.4%)	0.159
Vomiting		0 (0%)	15 (1.0%)	1.000
Diarrhea		2 (5.1%)	52 (3.3%)	0.381
Others		3 (7.7%)	38 (2.4%)	0.075
Underlying disease				
Hypertension		13 (33.3%)	471 (30.1%)	0.724
Use of ARB		7 (17.9%)	280 (17.9%)	1.000
Use of ACEI		1 (2.6%)	31 (2.0%)	0.549
Dyslipidemia		11 (28.2%)	423 (27.0%)	0.856
Diabetes		10 (25.6%)	296 (18.9%)	0.302
Type 1 diabetes		0 (0%)	22 (1.4%)	1.000
Type 2 diabetes		10 (25.6%)	274 (17.5%)	0.203
Hyperuricemia		1 (2.6%)	106 (6.8%)	0.513
Cerebrovascular disease		1 (2.6%)	26 (1.7%)	0.489
Heart disease		4 (10.3%)	129 (8.2%)	0.559
Thromboembolism		1 (2.6%)	18 (1.2%)	0.375
Lung disease		5 (12.8%)	84 (5.4%)	0.061
Liver disease		2 (5.1%)	37 (2.4%)	0.245
Kidney disease		1 (2.6%)	58 (3.7%)	1.000
Immunological disease		2 (5.1%)	87 (5.6%)	1.000
Group	Patient	29 (74.4%)	959 (61.3%)	0.113
	Doctor/nurse	10 (25.6%)	494 (31.6%)	
	Control	0 (0%)	111 (7.1%)	
Site	Clinic	32 (82.1%)	1253 (80.1%)	0.130
	Hospital	6 (15.4%)	306 (19.6%)	
	Clinic/hospital	1 (2.6%)	5 (0.3%)	
Medical institutions designated for COVID-19	Applicable	1 (2.6%)	116 (7.4%)	0.358
	Not applicable	38 (97.4%)	1448 (92.6%)	
		positive (n = 10)	negative (n = 494)	P
Occupation	Doctor	2 (20.0%)	215 (43.5%)	0.200
	Nurse	8 (80.0%)	279 (56.5%)	
In charge of	Outpatient	7 (70.0%)	373 (75.5%)	0.473
	General ward	1 (10.0%)	68 (13.8%)	
	Others	2 (20.0%)	53 (10.7%)	

Data are mean ± SD or n(%).

Data on the nominal scale were tested using the Fisher's exact test, and continuous variables were tested using the Student's test.



study. Therefore, conceivably, our kit will produce fewer false positive results than other kits [31].

In the present survey, antibody prevalence in the control group was 0%, and that in the doctor/nurse group and in the patient group was approximately 2%. In the survey by SoftBank Group, antibody prevalence was higher among healthcare workers although presence/absence of underlying disease was unknown. These results suggest that the infection is spreading in medical institutions among both doctors and patients.

Regarding other countries, antibody prevalence in the United States was 1.5% in Santa Clara County [12] and 4.06% in Los Angeles County of California state [13] and 14.9% in New York State [14]. In 90 Brazilian cities the sample size of which was 200 or more, overall antibody prevalence was 1.4% [15]. In Germany, antibody prevalence rate of 14% has been reported [16]. Although antibody prevalence was exceptionally high in Germany and New York State, it was similar in other cities, ranging from 1.4% to 4.06%. As of June 30, the infection rate was 194,259/83,517,000 (0.2%) in Germany [32,33] and 393,454/19,453,561 (2.0%) in New York [34,35]. It was 1502/9,222,162 (0.02%) in Kanagawa Prefecture [36,37], indicating the epidemic there is smaller compared to Germany and New York. This may explain why there are fewer antibody-positive individuals due to subclinical infections. There is also a 10-fold difference between Germany and New York in infection rate. However, subclinical infections are similar. Factors for this are not clear.

In the present survey, we conducted a questionnaire survey about possible factors for subclinical infections. However, none of them was found to be a significant factor for antibody-positive status.

We thought that overseas travel and contact with overseas travelers might be factors for subclinical infections. However, these were not found to be significant factors. None of the antibody-positive subjects had a history of overseas travel in 2020, while there were 85 overseas travelers in the negative group including those who traveled to countries where the novel coronavirus epidemic occurred. Regarding contact with overseas travelers or travelers who visited Japan in 2020, the positive group tended to have fewer contacts.

In terms of crowded places/close-contact settings/closed spaces, we thought that frequent use of trains might be a factor for subclinical infections. However, in both groups, the same proportion (approximately 20%) of the subjects used trains 5 times a week or more. During rush hour, trains have passengers more than 140% of their capacity in 15 of 23 train lines in Kanagawa, which adjoins Tokyo [38]. The government has issued an alert to avoid crowded places/close-contact settings/closed spaces. On the basis of the results from the present survey, however, it is unlikely that packed trains have any influence.

No influence of the presence/absence of individuals infected with the novel coronavirus in the living area was also detected. Combined with the fact that the use of trains had no influence on subclinical infection, it is conceivable that the use of a mask to prevent droplet infection was effective [39].

BCG vaccination was not a factor for antibody-positive status, either. High vaccination coverage in Japan might have made it difficult to see difference. It was confirmed that none of the antibody-positive subjects was diagnosed with influenza, that COVID-19 and influenza were distinguished, and that no one had influenza and COVID-19 concurrently.

Regarding underlying disease, 5 subjects in the positive group (12.8%) and 84 subjects in the negative group (5.4%) had lung disease. The difference was not significant. Lung disease was not a significant factor in multivariate analysis, either. However, it is a risk factor for severe disease once infection is established.

Therefore, caution will be needed. Since SARS-CoV-2 binds to ACE2 (angiotensin-converting enzyme 2) receptor on the cell surface and then enters the cell [40], we analyzed relationships to the use of ACEI and ARB. However, no specific tendency was observed. There was a report that ACEI or ARB does not pose a risk for severe disease [41]. We also think that they have no effect on subclinical infection. The positive rate of SARS-CoV-2 antibodies in medical institutions designated for COVID-19 was 0.90% (1/117), and other medical institutions, 2.6% (38/1486). There was no significant difference. COVID-19 patients with no symptoms are more likely to have brought the virus into hospitals than patients with COVID-19 positive transmit the virus to ordinary patients in hospitals. Besides, some patients were found in an elementary school and nursery school near two medical institutions with high positive rates of antibodies (6 out of 20 cases, 3 out of 20 cases), and these schools were closed. This result seems to have clinical significance in estimating the transmission status of infection in the area.

There was a report with data from 149 patients demonstrating that neutralizing antibody titers in convalescent plasma vary widely and most of them are low [42]. A study conducted in China confirmed that antibody levels decline dramatically after 2–3 months in approximately 90% of individuals [43]. If the same is true for Japanese, it would be difficult to know the current status of subclinical infections. As a follow-up to the present study, we started following up antibody-positive subjects and will report the results.

#### 4.1. Limitation

The present study has several limitations. One of them is that the population consisted of doctors, nurses, and individuals who regularly visit medical institutions. It is also undeniable that 111 subjects in the control group who had no underlying disease were different from other groups in terms of age, gender, and other characteristics. The present study was biased because it was a survey conducted with outpatients of medical institutions. Therefore, the subclinical infection rate in the entire population of Kanagawa Prefecture cannot be deduced from the results of the present study. There were only 12 minors. Therefore, the current status of the younger generation is still unknown. Also, in this study, the subjects were limited to patients with asymptomatic. The same result may not be obtained when targeting symptomatic people. In addition, it's difficult to simply compare the research results with other reports since many different test kits have been widely distributed now. The study will enhance more accuracy in the future if multiple assay kits to detect antibodies against SARS-CoV-2 are used at the same time to verify the results.

## 5. Conclusions

In Kanagawa Prefecture, Japan, antibody prevalence in the present survey at medical institution was 2.4%. After adjustment for age, sex, and the antibody prevalence in the control group, antibody prevalence was 2.7% in the patient group and 2.1% in the doctor/nurse group. These are higher than those in Tokyo and in Osaka measured by the government suggesting that subclinical infections are occurring more frequently than expected. No background factor that influenced antibody-positive status due to subclinical infection was identified.

#### Author contribution

Conceived and designed the experiments: IM, NH, HD, MN, AK. Enrolled patients: IM, NH, NK, YW, FE, YM, TN, TK, YO, AK. Analyzed

the data: IM. Wrote the first draft of the manuscript: IM, AK. Contributed to the writing of the manuscript: IM, AK. All authors agreed with the manuscript's results and conclusions. All authors have read, and confirm that they meet, ICMJE criteria for authorship.

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## Ethical approval and consent to participate

This study was registered with the Clinical Trials Registry (<https://www.umin.ac.jp/>; UMIN000040333) and was undertaken in accordance with the study protocol, the Declaration of Helsinki and the Ethical Guidelines for Clinical Studies of the Japanese Ministry of Health, Labor, and Welfare. This study was approved by the Ethics Review Board of the Kanagawa Physicians Association (April 27, 2020). The all participants provided written informed consent before participation.

## Availability of data and material

The biochemical data used to support the findings of this study are available from the corresponding author upon request.

## Declaration of competing interest

The authors declare no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jiac.2020.09.005>.

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