Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Original Article

Epidemiological study using IgM and IgG antibody titers against SARS-CoV-2 in The University of Tokyo, Japan (UT-CATS)☆

Akihisa Mitani [a,*,1], Kensuke Hamada [a,1], Naoyuki Yoshikawa [b], Yoshifumi Morita [b], Takeshi Horie [a], Yukiko Inoue [a], Minako Saito [a], Takashi Ishii [a], Mitsuhiro Sunohara [a], Ryota Takahashi [a], Noriko Emoto [a], Nahoko Nishimoto [a], Yoko Murano [a], Sachiko Okazaki [a], Shoko Tateishi [a], Atsushi Yao [a], Takuya Shimura [b], Makoto Kurano [b], Yutaka Yatomi [b], Shintaro Yanagimoto [a]

[a] Division for Health Service Promotion, The University of Tokyo, Japan
[b] Department of Clinical Laboratory, The University of Tokyo Hospital, Japan

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ABSTRACT

Introduction: The worldwide pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has continued to date. Given that some of the patients with coronavirus disease 2019 (COVID-19) are asymptomatic, antibody tests are useful to determine whether there is a previous infection with SARS-CoV-2. In this study, we measured IgM and IgG antibody titers against SARS-CoV-2 in the serum of asymptomatic healthy subjects in The University of Tokyo, Japan.

Methods: From June 2020, we recruited participants, who were students, staff, and faculty members of The University of Tokyo in the project named The University of Tokyo COVID-19 Antibody Titer Survey (UT-CATS). Following blood sample collection, participants were required to answer an online questionnaire about their social and health information. We measured IgG and IgM titers against SARS-CoV-2 using iFlash-SARS-CoV-2 IgM and IgG detection kit which applies a chemiluminescent immunoassay (CLIA) for the qualitative detection.

Results: There were 6609 volunteers in this study. After setting the cutoff value at 10 AU/mL, 32 (0.48%) were positive for IgG and 16 (0.24%) for IgM. Of six participants with a history of COVID-19, five were positive for IgG, whereas all were negative for IgM. The median titer of IgG was 0.40 AU/mL and 0.39 AU/mL for IgM. Both IgG and IgM titers were affected by gender, age, smoking status, and comorbidities.

Conclusions: Positive rates of IgG and IgM titers were relatively low in our university. Serum levels of these antibodies were affected by several factors, which might affect the clinical course of COVID-19.

1. Introduction

Since December 2019, a novel infectious disease, the coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread resulting in a global health crisis. In Japan, the first COVID-19 patient was confirmed in January 2020, and the first infection wave died down at the end of April after the declaration of a state of emergency by the Japanese government. However, the number of patients with COVID-19 began rising again in late June, marking the second wave, which peaked in early August [1]. Japan enjoyed a temporary lull until it found itself in the middle of a third wave of the pandemic in early November. According to the Ministry of Health, Labor and Welfare of Japan, to date (early February 2021), there have been approximately 400,000 confirmed cases of COVID-19 and 6000 deaths nationwide [2]. In Tokyo, where about one tenth of the entire population in Japan lives, there were more than 100,000 confirmed cases and over 1000 deaths [3].

SARS-CoV-2 infection is confirmed by a positive result of reverse

Abbreviations: COVID-19, coronavirus disease 2019; DL, dyslipidemia; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

☆ All authors meet the ICMJE authorship criteria.

* Corresponding author. Hongo 7-3-1, Bunkyo-ku, Tokyo, 113-0033, Japan.
E-mail address: mitania-tky@umin.ac.jp (A. Mitani).

[1] Contributed equally.

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Table 1
Profiles of participants.

	Total		Male		Female		p-value
n	6609		3860		2749		
Age (year, median [range], n = 6609 [3860/2749])[a]	36	[18–83]	33	[18–83]	40	[18–68]	<0.0001
−19 (%)	419	(6.34)	282	(7.31)	137	(4.98)	
20–29 (%)	1796	(27.18)	1243	(32.20)	553	(20.12)	
30–39 (%)	1584	(23.97)	959	(24.84)	625	(22.74)	
40–49 (%)	1445	(21.86)	673	(17.44)	772	(28.08)	
50–59 (%)	1007	(15.24)	475	(12.31)	532	(19.35)	
60- (%)	358	(5.42)	228	(5.91)	130	(4.73)	
Smoking (n = 6002 [3548/2544])[a]							<0.0001
Never (%)	5052	(84.17)	2815	(81.41)	2237	(87.93)	
Former (%)	715	(11.91)	455	(13.16)	260	(10.22)	
Current (%)	235	(3.92)	188	(5.44)	47	(1.85)	
Body temperature (°C, mean ± SD, n = 5159 [2824/2335])	36.37	±0.29	36.39	±0.28	36.37	±0.31	0.05948
Healthcare professional (n = 5383 [3010/2373])[a]							<0.0001
Yes (%)	859	(15.96)	433	(14.39)	426	(17.95)	
No (%)	4524	(84.04)	2577	(85.61)	1947	(82.05)	
Working place (n = 5161 [2826/2335])							
Hongo campus (%)	3217	(62.33)	1762	(62.35)	1455	(62.31)	1.0000
Komaba campus (%)	1362	(26.39)	757	(26.79)	605	(25.91)	0.4967
Kashiwa campus (%)	634	(12.28)	353	(12.49)	281	(12.03)	0.6490
Others (%)	76	(1.47)	42	(1.49)	34	(1.46)	1.0000
Comorbidity (n = 6054 [3497/2557])							
Any (%)	1247	(20.60)	693	(19.82)	554	(21.67)	0.0845
Hypertension (%)[a]	250	(4.13)	175	(5.00)	75	(2.93)	<0.0001
Dyslipidemia (%)[a]	193	(3.19)	127	(3.63)	66	(2.58)	0.0261
Diabetes mellitus (%)[a]	57	(0.94)	43	(1.23)	14	(0.55)	0.0099
Asthma (%)	97	(1.60)	54	(1.54)	43	(1.68)	0.7511
Allergic disease except for asthma (%)	277	(4.58)	171	(4.89)	106	(4.15)	0.1912
Past medical history (n = 6054 [3497/2557])							
COVID-19 (%)	6	(0.10)	3	(0.09)	3	(0.12)	1.0000
Medication (n = 5161 [2826/2335])							
Immunosuppressant (%)[a]	22	(0.43)	7	(0.25)	15	(0.64)	0.0331
Antihypertensive agent (%)[a]	192	(3.72)	121	(4.28)	71	(3.04)	0.0232

Numbers after total n indicate [male/female]. P-values are calculated from statistical tests between genders.
[a] Indicates factors with significant difference.

transcription polymerase chain reaction, and the patients often suffer from various symptoms such as fever, cough, and dyspnea. However, it has been reported that approximately 40%–45% of patients with COVID-19 are asymptomatic [4]. Therefore, some of them may not be diagnosed, resulting in an underestimation of the number of patients. Given that an epidemiological study to estimate the rate of infection of SARS-CoV-2 for infection control, antibody or serology tests, which look for antibodies in the blood to determine if there is a previous infection with SARS-CoV-2 are of increasing importance. Some surveillance reports have been already published in Tokyo [5,6], but they showed little data about the characteristics of subjects.

iFlash-SARS-CoV-2 IgM and IgG detection kit is the commercially available antibody test kit, and has been well validated in symptomatic COVID-19 cases [7–11]. The sensitivity and specificity of IgM were reported to be 70–96% and 84–96%, and those of IgG were 90–97% and 92–99%. In this study, we measured serum IgM and IgG antibody titers against SARS-CoV-2 in asymptomatic healthy subjects in The University of Tokyo, Japan using this kit, and investigated the relationship between seropositivity and participant characteristics including comorbidities and the past history of COVID-19.

2. Methods

2.1. Study design and population

The participants included students, staff, and faculty members of The University of Tokyo from June 2020 to October 2020. We used the health service center's web site to recruit the participants. We also set a booth for this research at each health checkup venue. They were included in the study after a consent form was signed. Blood samples were collected at health service centers or health checkup venues in the Hongo, Komaba, and Kashiwa campuses of The University of Tokyo, or The University of Tokyo Hospital. The Hongo and Komaba campuses are located in Tokyo metropolitan area, and the Kashiwa campus is in Kashiwa city, a commuter town in Greater Tokyo. The University of Tokyo Hospital is located within the Hongo area. The project was named The University of Tokyo COVID-19 Antibody Titer Survey (UT-CATS).

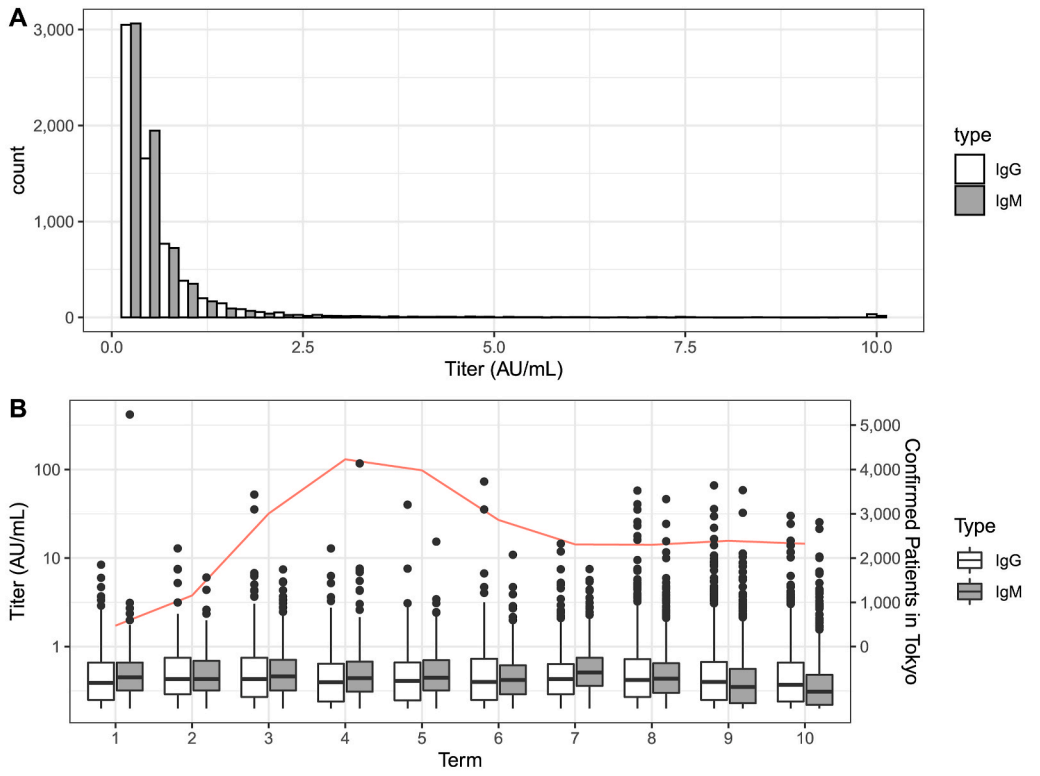
Participants are required to answer the online questionnaire on gender, age, occupation (healthcare professional or not), working place (campuses), comorbidities and past medical history with COVID-19, medication (immunosuppressant and antihypertensive agents), smoking history (never, former, or current smoker), and physical condition at blood collection. We analyzed the collected data after anonymization.

In this study, we used data from participants from whom blood samples were collected between June 11, 2020 and October 28, 2020. Based on the date of blood collection, we divided participants into 10 groups, term 1 to 10, in chronological order for every 14 days. In case of no answer about occupation in the online questionnaire, we considered that the participants were not healthcare professionals when they were confirmed as students.

The study protocol was approved by The University of Tokyo, Clinical Research Review Board (Registration number: 2020052NI).

2.2. Detection of SARS-CoV-2 specific serum antibodies

We measured the serum IgM and IgG antibody titers against SARS-CoV-2 using the quantitative iFlash 3000 chemiluminescent immunoassay from YHLO Biotechnology Company, Ltd. (Shenzhen, China), using magnetic beads coated with SARS-CoV-2 nucleocapsid protein and spike protein. Data below the detection limit (0.20 AU/mL) were considered as 0.20 in the subsequent analyses.



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Term		1	2	3	4	5	6	7	8	9	10	Total
Start		11-Jun	25-Jun	9-Jul	23-Jul	6-Aug	20-Aug	3-Sep	17-Sep	1-Oct	15-Oct	
End		24-Jun	8-Jul	22-Jul	5-Aug	19-Aug	2-Sep	16-Sep	30-Sep	14-Oct	28-Oct	
IgM (AU/mL) (%)	0.20-0.99	175 (88.83)	135 (90.00)	707 (87.07)	259 (88.7)	179 (87.75)	251 (89.96)	363 (85.01)	1281 (89.02)	1297 (89.94)	1288 (94.22)	5935 (89.80)
	1.00-1.99	18 (9.14)	11 (7.33)	87 (10.71)	23 (7.88)	18 (8.82)	22 (7.89)	49 (11.48)	118 (8.20)	108 (7.49)	52 (3.80)	506 (7.66)
	2.00-2.99	2 (1.02)	2 (1.33)	9 (1.11)	2 (0.68)	4 (1.96)	3 (1.08)	7 (1.64)	19 (1.32)	16 (1.11)	11 (0.80)	75 (1.13)
	3.00-3.99	1 (0.51)	0 (0.00)	5 (0.62)	1 (0.34)	2 (0.98)	1 (0.36)	3 (0.70)	9 (0.63)	7 (0.49)	4 (0.29)	33 (0.50)
	4.00-4.99	0 (0.00)	1 (0.67)	1 (0.12)	2 (0.68)	0 (0.00)	1 (0.36)	2 (0.47)	5 (0.35)	2 (0.14)	4 (0.29)	18 (0.27)
	5.00-5.99	0 (0.00)	0 (0.00)	2 (0.25)	1 (0.34)	0 (0.00)	0 (0.00)	2 (0.47)	2 (0.14)	2 (0.14)	1 (0.07)	10 (0.15)
	6.00-6.99	0 (0.00)	1 (0.67)	0 (0.00)	1 (0.34)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.14)	2 (0.15)	6 (0.09)
	7.00-7.99	0 (0.00)	0 (0.00)	1 (0.12)	2 (0.68)	0 (0.00)	0 (0.00)	1 (0.23)	0 (0.00)	1 (0.07)	1 (0.07)	6 (0.09)
	8.00-8.99	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.21)	0 (0.00)	3 (0.05)
	9.00-9.99	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)	0 (0.00)	0 (0.00)	1 (0.02)
	10.00-	1 (0.51)	0 (0.00)	0 (0.00)	1 (0.34)	1 (0.49)	1 (0.36)	0 (0.00)	4 (0.28)	4 (0.28)	4 (0.29)	16 (0.24)
IgG (AU/mL) (%)	0.20-0.99	173 (87.82)	127 (84.67)	692 (85.22)	248 (84.93)	178 (87.25)	234 (83.87)	379 (88.76)	1222 (84.92)	1247 (86.48)	1184 (86.61)	5684 (86.00)
	1.00-1.99	13 (6.60)	15 (10.00)	85 (10.47)	29 (9.93)	21 (10.29)	32 (11.47)	32 (7.49)	149 (10.35)	130 (9.02)	132 (9.66)	638 (9.65)
	2.00-2.99	5 (2.54)	3 (2.00)	23 (2.83)	9 (3.08)	2 (0.98)	7 (2.51)	9 (2.11)	29 (2.02)	26 (1.80)	24 (1.76)	137 (2.07)
	3.00-3.99	3 (1.52)	1 (0.67)	4 (0.49)	2 (0.68)	1 (0.49)	1 (0.36)	1 (0.23)	13 (0.90)	13 (0.90)	12 (0.88)	51 (0.77)
	4.00-4.99	1 (0.51)	0 (0.00)	2 (0.25)	0 (0.00)	0 (0.00)	2 (0.72)	2 (0.47)	8 (0.56)	8 (0.55)	6 (0.44)	29 (0.44)
	5.00-5.99	1 (0.51)	1 (0.67)	1 (0.12)	1 (0.34)	0 (0.00)	0 (0.00)	1 (0.23)	8 (0.56)	3 (0.21)	0 (0.00)	16 (0.24)
	6.00-6.99	0 (0.00)	0 (0.00)	3 (0.37)	2 (0.68)	0 (0.00)	1 (0.36)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)	6 (0.09)
	7.00-7.99	0 (0.00)	2 (1.33)	0 (0.00)	0 (0.00)	1 (0.49)	0 (0.00)	1 (0.23)	2 (0.14)	1 (0.07)	0 (0.00)	8 (0.12)
	8.00-8.99	1 (0.51)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.28)	0 (0.00)	5 (0.08)
	9.00-9.99	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)	2 (0.14)	0 (0.00)	3 (0.05)
	10.00-	0 (0.00)	1 (0.67)	2 (0.25)	1 (0.34)	1 (0.49)	2 (0.72)	2 (0.47)	7 (0.49)	8 (0.55)	8 (0.59)	32 (0.48)

Fig. 1. Distribution of IgG and IgM titers
(A) Histogram of IgG and IgM titers. Data over 10.0 AU/mL are considered as 10.0 AU/mL in this histogram. (B) (C). The timeline of distributions. Red line indicates the number of confirmed patients in Tokyo.

2.3. Statistical analysis

Brunner–Munzel tests (2 groups) or Kruskal–Wallis tests (3 or more groups) were used for comparisons of age and titers of IgG and IgM, and *t*-test for body temperature. Fisher's exact tests were used for categorical variables that included values below 10, and chi-square tests for other categorical variables.

We performed all statistical analyses with the use of R 4.0.3 [12] with "lawstat" [13] and "tidyverse" [14] packages. The results are expressed as the median [range]. All tests were two-tailed, and a p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Characteristic of participants

From June 11, 2020 to October 28, 2020, 6609 participants were included in the study, of which 3860 (58.40%) were male and 2749 (41.60%) were female. The profiles of the participants are summarized in Table 1. The median age of female participants (40 years) was significantly higher than that of males (36 years) ($p < 0.0001$). The ratio of never a smoker or healthcare professionals was also higher in females than in males (87.93% vs 81.41% and 17.95% vs 14.39% respectively).

Approximately 21% had at least one comorbidity and there was no significant difference between genders. However, the rates of participants with hypertension (HT), dyslipidemia (DL), and diabetes mellitus (DM) were significantly higher in males. Six participants reported histories of COVID-19 with no significant difference between genders (3 vs. 3). As for medication, 22 participants (0.43%) were on an immunosuppressant, showing a significantly higher rate in females ($p = 0.0331$). Conversely, the rate of participants taking antihypertensive agent was significantly higher in males ($p = 0.0232$).

3.2. Distribution of IgG and IgM titers

Fig. 1A shows the distribution of IgG and IgM titers in total. Both IgG

Table 2
Profiles of participants with IgG titer or IgM titer over cutoff.

	IgM ≥10 AU/mL		p-value	IgG ≥10 AU/mL		p-value	Total	
n	16			32			6609	
Gender (Male/Female)	8/8		0.6132	18/14		0.9466	3860/2749	
Age (year, median [range])	46	[28–67]	0.0034	38	[18–75]	0.6477	36	[18–83]
	(n = 16 [8/8])			(n = 32 [18/14])			(n = 6609 [3860/2749])	
Smoking			0.1532			0.6813		
Never (%)	12	(75.00)		25	(92.59)		5052	(84.17)
Former (%)	2	(12.50)		2	(7.41)		715	(11.91)
Current (%)	2	(12.50)		0	(0.00)		235	(3.92)
	(n = 16 [8/8])			(n = 27 [14/13])			(n = 6002 [3548/2544])	
Body temperature (°C, mean ± SD)	36.30	±0.36	0.4454	36.37	±0.43	0.8198	36.37	±0.29
	(n = 13 [7/6])			(n = 24 [12/12])			(n = 5159 [2824/2335])	
Healthcare professional			1.0000			1.0000		
Yes (%)	2	(15.38)		3	(12.50)		859	(15.96)
No (%)	11	(84.62)		21	(87.50)		4524	(84.04)
	(n = 13 [7/6])			(n = 24 [12/12])			(n = 5383 [3010/2373])	
Working place			0.7048			0.7348		
Hongo campus (%)	9	(69.23)		13	(54.17)		3217	(62.33)
Komaba campus (%)	3	(23.08)		7	(29.17)		1362	(26.39)
Kashiwa campus (%)	3	(23.08)		4	(16.67)		634	(12.28)
Others (%)	0	(0.00)		0	(0.00)		76	(1.47)
	(n = 13 [7/6])			(n = 24 [12/12])			(n = 5161 [2826/2335])	
Comorbidity								
Any (%)	7	(43.75)	0.0313	4	(13.79)	0.4915	1247	(20.60)
Hypertension (%)	1	(6.25)	0.4916	1	(3.45)	1.0000	250	(4.13)
Dyslipidemia (%)	0	(0.00)	1.0000	0	(0.00)	1.0000	193	(3.19)
Diabetes mellitus (%)	0	(0.00)	1.0000	1	(3.45)	0.2431	57	(0.94)
Asthma (%)	0	(0.00)	1.0000	0	(0.00)	1.0000	97	(1.60)
Allergic disease except for asthma (%)	2	(12.50)	0.1657	1	(3.45)	1.0000	277	(4.58)
	(n = 16 [8/8])			(n = 29 [15/14])			(n = 6054 [3497/2557])	
Past medical history								
COVID-19 (%)	0	(0.00)	1.0000	5	(17.24)	<0.0001	6	(0.10)
	(n = 16 [8/8])			(n = 29 [15/14])			(n = 6054 [3497/2557])	
Medication								
Immunosuppressant (%)	1	(7.69)	0.0563	0	(0.00)	1.0000	22	(0.43)
Antihypertensive agent (%)	2	(15.38)	0.0832	1	(4.17)	0.5985	192	(3.72)
	(n = 13 [7/6])			(n = 24 [12/12])			(n = 5161 [2826/2335])	

Numbers after total n indicate [male/female]. P-values are calculated from statistical tests between over and under cutoff groups.

and IgM had similar distributions: approximately 90% were less than 1.00 AU/mL and more than 95% were less than 2.00 AU/mL. There was no correlation found between IgG and IgM titers (r = 0.0389, Supplementary Fig. 1). Compared in time series, no significant elevation was found, despite of the second wave peaking in term 4 (Fig. 1B). Therefore, we decided to analyze the data altogether during this period (just before the beginning of the third wave).

3.3. Participants with elevated IgG and IgM titers

According to the manual provided by the manufacturer, both cutoff values for IgG and IgM titers are 10 AU/mL. Thirty-two participants (0.48%) showed IgG titers above this cutoff, whereas the IgM titers of 16 participants (0.24%) were over the cutoff (Fig. 1C). The characteristics of these participants are summarized in Table 2. There was no significant difference between the elevated IgG and any factors including gender, age, smoking history, comorbidities, and working places, with the exception of a close relationship between the elevated IgG and a history of COVID-19. As for IgM, participants with an elevated IgM titer tended to be older and were more disposed to having any comorbidities.

Nakano et al. analyzed the antibody titers of Japanese patients with symptomatic COVID-19, and suggested that the cutoff values in Japan might be lower than the manufacturer's reported cutoff [15]. Using the cutoff value of 5AU/mL, the number of positive participants for IgM was 42 (0.64%), and one for IgG was 70 (1.06%). We also investigated the characteristics of the positive participants with this cutoff (Supplementary Table 1). There was no obvious difference compared to the correlations using the cutoff of 10 AU/mL.

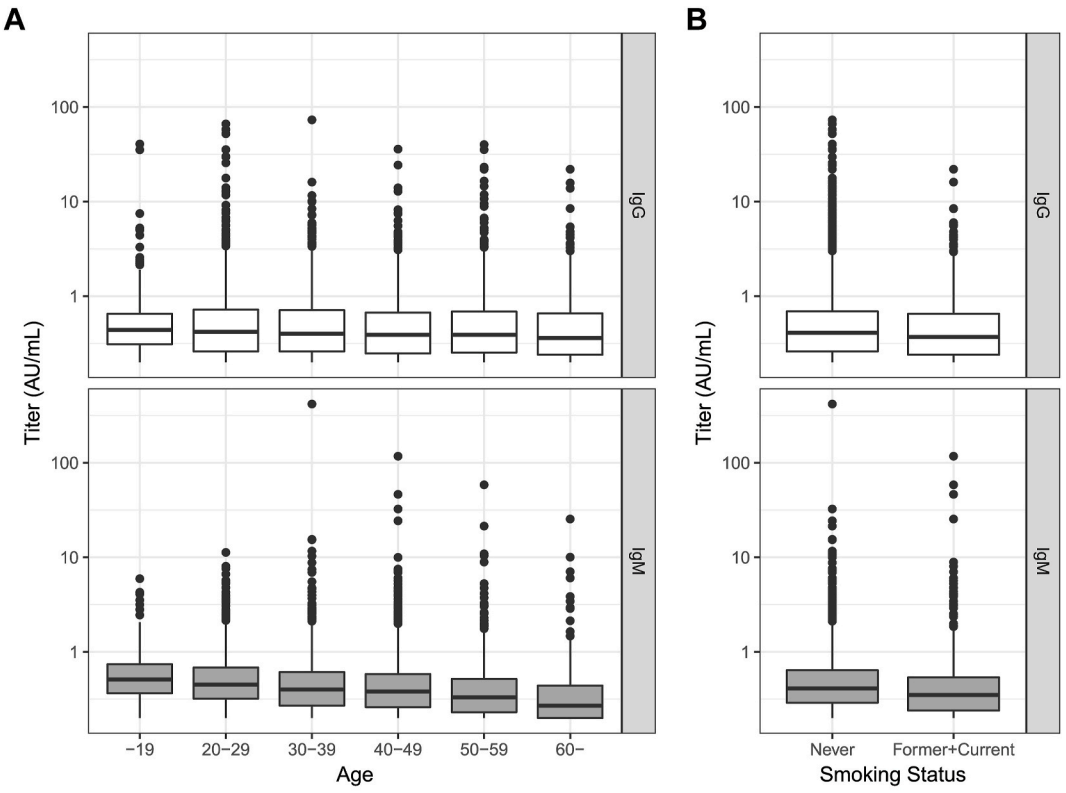
3.4. Participants with a history of COVID-19

As described in Table 2, six participants (0.09%) had a history of COVID-19. Their gender, IgG and IgM titers, duration between diagnosis of COVID-19 and blood collection, occupation, and working place are shown in Supplementary Table 2. Five participants (except for participant no. 4), all of whom had their blood collected within 6 months of the diagnosis, had increased IgG titers over 10 AU/mL, whereas the maximum IgM titer among these patients was 1.63 AU/mL. Only participant no. 4, whose IgG titer was not elevated over 10 AU/mL, had his blood collected over 6 months after the diagnosis (Supplementary Fig. 2).

3.5. The factors effecting IgG and IgM titers

We then analyzed the IgG and IgM titers of the all participants in a quantitative manner. The median titers were 0.40 AU/mL and 0.39 AU/mL for IgG and IgM in total, respectively. Female participants had significantly increased titers for both IgG (0.43 AU/mL vs 0.38 AU/mL, $p < 0.001$) and IgM (0.47 AU/mL vs 0.35 AU/mL, $p < 0.0001$) (Fig. 2C).

At first, IgG and IgM titers were compared by age or smoking status (Fig. 2). Both IgG and IgM titers were found to be decreased significantly with age ($p < 0.01$ for IgG and $p < 0.0001$ for IgM) (Fig. 2A and C). After stratification by gender, the IgM titer remained significantly decreased in both genders, whereas the IgG titer decrease was not significant in the female group. Conversely, compared according to smoking status, IgG and IgM titers were significantly decreased in the former +current smokers group (Fig. 2B, $p < 0.001$ for IgG and $p < 0.0001$ for IgM) (Fig. 2B and C). Analysis using only the male group also had the same tendency.



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IgM (AU/mL)	Total			Male			Female		
	median	range	p-value	median	range	p-value	median	range	p-value
Total (n = 6,609 [3,860/2,749])	0.39	[0.20-417.65]		0.35	[0.20-117.20]		0.47	[0.20-417.65]	
Age (n = 6,609 [3,860/2,749])			<0.0001			<0.0001			<0.0001
-19	0.51	[0.20-5.94]		0.44	[0.20-4.27]		0.66	[0.204-5.94]	
20-29	0.45	[0.20-11.26]		0.42	[0.20-11.26]		0.53	[0.20-7.61]	
30-39	0.40	[0.20-417.65]		0.34	[0.20-15.32]		0.50	[0.20-417.65]	
40-49	0.38	[0.20-117.20]		0.30	[0.20-117.20]		0.45	[0.20-46.31]	
50-59	0.33	[0.20-58.43]		0.28	[0.20-58.43]		0.37	[0.20-21.39]	
60-	0.27	[0.20-25.39]		0.25	[0.20-25.39]		0.34	[0.20-7.05]	
Smoking (n = 6,002 [3,548/2,544])			<0.0001			<0.0001			0.0851
Never	0.41	[0.20-417.65]		0.37	[0.20-24.31]		0.47	[0.20-417.65]	
Former + Current	0.35	[0.20-117.20]		0.31	[0.20-117.20]		0.46	[0.20-46.31]	

IgG (AU/mL)	Total			Male			Female		
	median	range	p-value	median	range	p-value	median	range	p-value
Total (n = 6,609 [3,860/2,749])	0.40	[0.20-73.09]		0.38	[0.20-57.81]		0.43	[0.20-73.09]	
Age (n = 6,609 [3,860/2,749])			0.0070			0.0045			0.1746
-19	0.44	[0.20-40.61]		0.44	[0.20-40.61]		0.46	[0.20-4.99]	
20-29	0.42	[0.20-66.18]		0.41	[0.20-57.81]		0.44	[0.20-66.18]	
30-39	0.40	[0.20-73.09]		0.38	[0.20-16.06]		0.44	[0.20-73.09]	
40-49	0.39	[0.20-35.86]		0.37	[0.20-7.45]		0.42	[0.20-35.86]	
50-59	0.39	[0.20-40.06]		0.38	[0.20-21.95]		0.41	[0.20-40.06]	
60-	0.36	[0.20-21.98]		0.35	[0.20-21.98]		0.39	[0.20-15.74]	
Smoking (n = 6,002 [3,548/2,544])			<0.001			0.0053			0.2030
Never	0.41	[0.20-73.09]		0.39	[0.20-57.81]		0.43	[0.20-73.09]	
Former + Current	0.37	[0.20-21.98]		0.36	[0.20-21.98]		0.39	[0.20-5.76]	

Fig. 2. Comparisons of IgG and IgM titers according to age and smoking status. IgG and IgM titers were compared according to age (A and C) and smoking status (B and C). Numbers after total n indicate [male/female]. P-values in total are calculated from statistical tests between genders.

As for occupation, IgM titers of healthcare professionals increased significantly in total and in the female group, whereas IgG titers did not show any significant changes in all groups (Table 3). IgM titers differed significantly in the general group and in both genders according to the campuses they worked or studied, whereas IgG titers did not (Table 3).

The results of comparison according to comorbidities, past medical history with COVID-19, and medication are shown in Table 4. A history of COVID-19 had a significant correlation with an increased IgG titer, and tended to be also associated with an increased IgM titer although there is no significant difference. Furthermore, there were significant changes of IgM titers in several comorbidity groups: any, HT, DL, DM (except for the male group), and allergic diseases (except for the male group). All of these showed a decrease of titers in the disease groups. On the contrary, a significant change of IgG titer was found in DL (total and male group). There was no significant change in all groups compared with taking an immunosuppressant. Among the participants with HT, neither IgG nor IgM titers were changed by antihypertensive agents such as calcium channel blocker or angiotensin-converting enzyme inhibition/angiotensin II receptor blocker (Supplementary Table 3).

4. Discussion

In the present study, we measured IgG and IgM titers against SARS-CoV-2 of the members of one university in Tokyo, Japan, from the

Table 3
Titers stratified by occupation and working place.

IgM (AU/mL)	Total			Male			Female		
	median	range	p-value	median	range	p-value	median	range	p-value
Healthcare professionals			<0.001			0.6441			0.0004
Yes (n = 859 [433/426])	0.43	[0.20–417.65]		0.37	[0.20–24.31]		0.51	[0.20–417.65]	
No (n = 4524 [2577/1947])	0.40	[0.20–117.20]		0.37	[0.20–117.20]		0.46	[0.20–32.42]	
Working place			<0.0001			<0.0001			0.01823
Hongo campus (n = 3217 [1762/1455])	0.41	[0.20–417.65]		0.37	[0.20–117.20]		0.47	[0.20–417.65]	
Komaba campus (n = 1362 [757/605])	0.40	[0.20–32.42]		0.37	[0.20–24.31]		0.46	[0.20–32.42]	
Kashiwa campus (n = 634 [353/281])	0.36	[0.20–58.43]		0.31	[0.20–58.43]		0.45	[0.20–8.78]	
Others (n = 76 [42/34])	0.40	[0.20–1.55]		0.36	[0.20–1.48]		0.42	[0.20–1.55]	
IgG (AU/mL)	Total median	range	p-value	Male median	range	p-value	Female median	range	p-value
Healthcare professionals			0.0665			0.0576			0.3426
Yes (n = 859 [433/426])	0.38	[0.20–66.18]		0.35	[0.20–8.40]		0.42	[0.20–66.18]	
No (n = 4524 [2577/1947])	0.41	[0.20–73.09]		0.39	[0.20–57.81]		0.43	[0.20–73.09]	
Working place			0.1791			0.0929			0.8002
Hongo campus (n = 3217 [1762/1455])	0.39	[0.20–73.09]		0.37	[0.20–52.13]		0.42	[0.20–73.09]	
Komaba campus (n = 1362 [757/605])	0.41	[0.20–57.81]		0.41	[0.20–57.81]		0.42	[0.20–35.39]	
Kashiwa campus (n = 634 [353/281])	0.42	[0.20–29.64]		0.38	[0.20–29.64]		0.47	[0.20–10.70]	
Others (n = 76 [42/34])	0.44	[0.20–6.71]		0.47	[0.20–4.21]		0.41	[0.20–6.71]	

Numbers after total n indicate [male/female].

beginning of the second wave (mid-June 2020) to just before the third wave (the end of October 2020). We performed admission management and daily health condition monitoring using online reporting system, and no infection cluster had occurred in the university. During the study period, the distributions of IgG and IgM titers did not changed. Among the participants, six (0.09%) had a history of COVID-19. It has been reported that the cumulative number of patients with COVID-19 was 31,000 at the end of October 2020 [16], which represents 0.22% of the population of Tokyo.

The manufacturer of the kit used in this study recommends to set the cutoff value at 10 AU/mL both for IgG and IgM, and previous reports have used this value [10,17]. Applying this cutoff to our results, the positive rate was 0.48% for IgG and 0.24% for IgM. The Ministry of Health, Labor and Welfare of Japan performed the IgG antibody survey at three different regions including Tokyo, using two commercial antibody tests provided by Abbott (IgG) and Roche (total Ig). According to the reported results, of 1971 participants from Tokyo, 6 (0.30%) were positive for Roche, 4 (0.20%) were positive for Abbott, and 2 (0.10%) were positive for both on June 2020 [5], and of 3399 participants from Tokyo, 60 (1.76%) were positive for Roche, 37 (1.09%) were positive for Abbott, and 31 (0.91%) were positive for both on December 2020 [6]. These results are comparable with our results. We concluded that the positive rates of IgG and IgM antibodies were still low before the third wave.

The number of participants with elevated IgG titers was 2.5 times greater than that of the participants with a history of COVID-19. We could not overemphasize the importance of the existence of asymptomatic patients with COVID-19, who have sometimes never been diagnosed. Five of six participants with a history of COVID-19 had an elevated IgG titer, and only one participant who did not show IgG titer elevation had his blood sample collected 195 days after the diagnosis. Previous reports have revealed that IgG against SARS-CoV-2 gradually declines and sometimes results in seronegative 6 months after infection [18,19], which is in corroboration with our findings. Therefore, we can conclude that our survey had a satisfying sensitivity, but it is essential to pay attention to the timing of blood sample collection because the pandemic continued for over one year worldwide.

As mentioned above, 32 participants (0.48%) had IgG titers above the cutoff value, and 16 participants (0.24%) for IgM. The participants with the positive IgG titers had no specific features compared to all participants, although the positive IgM titer seemed to be correlated with old age and having any comorbidity.

Subsequently, we performed quantitative analysis, which revealed a significant decrease of the IgM titer for several factors: gender (male), age, smoking, and comorbidities of HT, DL, and DM. The IgG titer was negatively correlated with comorbidities of DL, and positively with COVID-19. Interestingly, all of these except for COVID-19 have been reported as risk factors of severe illness from COVID-19 [20–26]. It has also been reported that whole IgM declines with age [27], which would affect IgM titer tendency.

Our results also suggest that occupation (healthcare professionals or not) and working place may affect the IgM titer. Considering the fact that most healthcare professionals work on the Hongo Campus, which includes The University of Tokyo Hospital, they may affect each other. The fact that the IgG titers of healthcare professionals were not increased would make us expect that infection prevention works sufficiently in The University of Tokyo Hospital, although it goes without saying that not all members of the hospital are not working at COVID-19 ward.

There are several limitations in this study. First, since only a small percentage of participants showed IgG or IgM titers over 10 AU/mL, we were unable to eliminate the possible confounding factors, such as age and comorbidities. Second, members of The University of Tokyo do not always reflect the population of Tokyo or Japan. As for SARS-CoV-2 infection, there has been no outbreak among the members of The University of Tokyo. Hence, it is impossible to generalize our results to the population of Tokyo or Japan. Third, we collected the data on comorbidities by self-reporting system using online questionnaire, and the definitions of the diseases were obscure. Forth, we could not exclude possible false positives of the antibody titer test. In general terms, low prevalence, often seen in a healthy population, is known to cause low positive predictive value. In this study, IgG and IgM titers seemed to be independently distributed, although it has been reported that they were both elevated in almost the same period in the serum of the patients with COVID-19 [28]. This fact also supports the possible contamination of false positive results especially for IgM. Finally, the exact significance of IgM and IgG titers is still unclear. Most of the significant differences in the present study are among values below the cutoffs, and there was some discrepancy in the results. On the other hand, there are some reports suggesting the higher or lower cutoff value [15,29,30], and the cutoff value itself for this emerging infectious disease is still under the validation process especially in asymptomatic subjects, which might be changed in future. Further study is needed to confirm the quantitative meanings of these values.

In conclusion, we found that the positive rates of IgG and IgM antibody titers against SARS-CoV-2 were low enough in our university. The

Table 4
Titers stratified by comorbidity, past medical history (COVID-19), and medication (immunosuppressant).

IgM (AU/mL)	Total			Male			Female		
	median	range	p-value	median	range	p-value	median	range	p-value
Comorbidity									
Any			<0.0001			<0.0001			<0.0001
YES (n = 1247 [693/554])	0.36	[0.20–58.43]		0.33	[0.20–58.43]		0.40	[0.20–46.31]	
NO (n = 4807 [2804/2003])	0.41	[0.20–417.65]		0.37	[0.20–117.20]		0.49	[0.20–417.65]	
Hypertension			<0.0001			<0.0001			<0.0001
YES (n = 250 [175/75])	0.30	[0.20–10.04]		0.28	[0.20–10.04]		0.34	[0.20–1.59]	
NO (n = 5804 [3322/2482])	0.41	[0.20–417.65]		0.37	[0.20–117.20]		0.48	[0.20–417.65]	
Dyslipidemia			<0.0001			0.0096			<0.001
YES (n = 193 [127/66])	0.34	[0.20–3.85]		0.29	[0.20–3.85]		0.37	[0.20–1.35]	
NO (n = 5861 [3370/2491])	0.40	[0.20–417.65]		0.36	[0.20–117.20]		0.48	[0.20–417.65]	
Diabetes Mellitus			0.0021			0.0732			0.0308
YES (n = 57 [43/14])	0.31	[0.20–1.5]		0.27	[0.20–1.5]		0.34	[0.20–0.81]	
NO (n = 5997 [3454/2543])	0.40	[0.20–417.65]		0.36	[0.20–117.20]		0.47	[0.20–417.65]	
Asthma			0.7745			0.9048			0.7345
YES (n = 97 [54/43])	0.39	[0.20–2.17]		0.38	[0.20–2.03]		0.43	[0.20–2.17]	
NO (n = 5957 [3443/2514])	0.40	[0.20–417.65]		0.36	[0.20–117.20]		0.47	[0.20–417.65]	
Allergic diseases			0.0067			0.2936			0.0037
YES (n = 277 [171/106])	0.36	[0.20–58.43]		0.34	[0.20–58.43]		0.39	[0.20–2.71]	
NO (n = 5777 [3326/2451])	0.40	[0.20–417.65]		0.36	[0.20–117.20]		0.48	[0.20–417.65]	
COVID-19			0.1644			0.5679			0.2349
YES (n = 6 [3/3])	0.83	[0.23–1.63]		0.75	[0.23–1.06]		0.90	[0.44–1.63]	
NO (n = 6048 [3494/2554])	0.40	[0.20–417.65]		0.36	[0.20–117.20]		0.47	[0.20–417.65]	
Immunosuppressant			0.8510			0.3380			0.2645
YES (n = 22 [7/15])	0.47	[0.20–10.04]		0.59	[0.20–10.04]		0.44	[0.20–1.52]	
NO (n = 5139 [2819/2320])	0.40	[0.20–417.65]		0.36	[0.20–117.20]		0.47	[0.20–417.65]	
IgG (AU/mL)	Total			Male			Female		
	median	range	p-value	median	range	p-value	median	range	p-value
Comorbidity									
Any			0.3462			0.4056			0.5561
YES (n = 1247 [693/554])	0.41	[0.20–66.18]		0.38	[0.20–21.98]		0.43	[0.20–66.18]	
NO (n = 4807 [2804/2003])	0.40	[0.20–73.09]		0.39	[0.20–57.81]		0.43	[0.20–73.09]	
Hypertension			0.2137			0.4990			0.3578
YES (n = 250 [175/75])	0.41	[0.20–21.98]		0.38	[0.20–21.98]		0.42	[0.20–5.23]	
NO (n = 5804 [3322/2482])	0.40	[0.20–73.09]		0.39	[0.20–57.81]		0.43	[0.20–73.09]	
Dyslipidemia			0.0171			0.3015			0.0217
YES (n = 193 [127/66])	0.36	[0.20–4.80]		0.38	[0.20–4.80]		0.35	[0.20–3.63]	
NO (n = 5861 [3370/2491])	0.41	[0.20–73.09]		0.39	[0.20–57.81]		0.43	[0.20–73.09]	
Diabetes Mellitus			0.7512			0.4657			0.8225
YES (n = 57 [43/14])	0.43	[0.20–21.95]		0.43	[0.20–21.95]		0.43	[0.20–3.74]	
NO (n = 5997 [3454/2543])	0.40	[0.20–73.09]		0.38	[0.20–57.81]		0.43	[0.20–73.09]	
Asthma			0.7182			0.4966			0.8436
YES (n = 97 [54/43])	0.39	[0.20–9.50]		0.36	[0.20–9.50]		0.42	[0.20–7.53]	
NO (n = 5957 [3443/2514])	0.40	[0.20–73.09]		0.39	[0.20–57.81]		0.43	[0.20–73.09]	
Allergic diseases			0.6649			0.6526			0.1657
YES (n = 277 [171/106])	0.42	[0.20–14.01]		0.40	[0.20–7.47]		0.46	[0.20–14.01]	
NO (n = 5777 [3326/2451])	0.40	[0.20–73.09]		0.38	[0.20–57.81]		0.43	[0.20–73.09]	
COVID-19			<0.0001			0.0021			<0.0001
YES (n = 6 [3/3])	13.41	[1.48–40.61]		21.95	[1.48–40.61]		12.85	[11.86–13.96]	
NO (n = 6048 [3494/2554])	0.40	[0.20–73.09]		0.38	[0.20–57.81]		0.43	[0.20–73.09]	
Immunosuppressant			0.0608			0.4950			0.3933
YES (n = 22 [7/15])	0.38	[0.20–21.98]		0.31	[0.20–0.86]		0.50	[0.21–0.84]	
NO (n = 5139 [2819/2320])	0.40	[0.20–73.09]		0.38	[0.20–57.81]		0.43	[0.20–73.09]	

Numbers after total n indicate [male/female].

serum levels of these antibodies are affected by several factors, including age, smoking habit, comorbidities and, needless to say, a past history of COVID-19, which might affect the clinical course of the disease.

Author contributions

AM, KH, MK, YY and SY conceived and designed the study. AM, KH, TH, YI and SY coordinated the study. AM, KH, TH, YI, MS, TI, MS, RT, NE, NN, YM, SO, ST, AY and SY took informed consent from a study participant. NY, YM and TS managed the bood sample collection, and performed the measurements of the titer. AM and KH wrote the manuscript. All authors have read, edited and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have 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.2021.06.008.

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