〈Regular Article〉

Changes in serum antibody titers after vaccination for COVID-19 and evaluation of post-vaccination health conditions

Shin OHIRA[1)], Takashi NAKANO[2)], Satoe UESUGI[3)], Yutaka KOUGUCHI[3)]
Masayo TAMURA[4)], Takako HIRAMATSU[5)], Mihoe ATARASHI[6)]
Tota NAKATSUKA[1)], Keita HIRATA[1)], Takaaki TANAKA[2)], Tomohiko KIMURA[7)]
Eiichiro KANDA[8)], Kaoru TOHYAMA[9)], Takanobu OTOMO[10)], Atsushi NAGAI[11)]

1) Department of Urology, Kawasaki Medical School
2) Department of Pediatrics, Kawasaki Medical School
3) Department of Clinical Laboratory, Kawasaki Medical School Hospital
4) Department of Clinical Laboratory, Kawasaki Medical School General Medical Center
5) Department of Nursing, Kawasaki Medical School Hospital
6) Department of Nursing, Kawasaki Medical School General Medical Center
7) Department of Diabetes, Endocrinology and Metabolism, Kawasaki Medical School
8) Medical Science, Kawasaki Medical School
9) Department of Laboratory Medicine, Kawasaki Medical School
10) Department of Molecular and Genetic Medicine, Kawasaki Medical School
11) Director of Kawasaki Medical School Hospital

ABSTRACT **Introduction**: The coronavirus disease 2019 (COVID-19) vaccine has preventive effects and high immunogenicity, but the outcomes of vaccination have not been widely reported. The goal of this study was to examine serum antibody titers before and after vaccination and to evaluate post-vaccination health conditions.

Methods: The subjects were 2,304 medical workers (mean age 41 years) at Kawasaki Gakuen who agreed to participate in the study and underwent COVID-19 vaccination, beginning in March 2021. Serum IgG antibody titers for SARS-CoV-2 spike protein were measured before the first vaccination and 4 weeks after the second vaccination. Health conditions were observed for 4 weeks after the second vaccination.

Results: The rates of seroconversion, seroprotection, and change in geometric mean antibody titer at 4 weeks after the second vaccination were 99.9%, 99.9%, and 2,685.5 (95% CI 587.8-5,319.2), respectively, suggesting high immunogenicity. After the first vaccination, pain, enlargement, and reddening occurred at the local injection site, and systemic side effects included fatigue, headache, physical pain, chill, nausea, and fever. After the second vaccination, the incidence of pain decreased, but those of other events increased. There were no serious side effects requiring hospitalization. In logistic regression analysis, sex, age, fever,

Corresponding author
Shin Ohira
Department of Urology, Kawasaki Medical School, 577 Matsushima, Kurashiki, 701-0192, Japan
Phone : 81 86 462 1111
Fax : 81 86 462 7897
E-mail: ohira@med.kawasaki-m.ac.jp

chill, and lymph node enlargement after the second vaccination were associated with a change in antibody titer.

Conclusions: Serum antibody titers suggested high immunogenicity of the COVID-19 vaccine and a health condition survey confirmed the safety of the vaccine. Systemic side effects may serve as an index of immunization (acquisition of antibody) by the vaccine.

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Key words：COVID-19 vaccine, Introduction of vaccination, Serum antibody titer, Health condition survey

INTRODUCTION

COVID-19 first appeared in December 2019 and the subsequent infection pandemic still continues worldwide. Vaccination plays an important role among preventive measures against COVID-19 infection. In Japan, the Pfizer mRNA COVID-19 vaccine was approved on February 14, 2021, and our facility began vaccination of medical workers as a priority group in March 2021. In clinical studies of mRNA COVID-19 vaccination, the incidence of pain at the injection site has been found to be highest among local side effects (70% to < 90%)[1-3] and the incidence of fatigue is highest for systemic side effects (50% to < 70%)[1-3]. The incidence of serious adverse events has not differed from that in control groups[1-3], suggesting the safety of the vaccine.

The preventive effect of the vaccine against COVID-19 infection is > 90%[1, 2, 4-6]. The seroconversion rate (SCR) and seroprotection rate (SPR) are 99-100%[7-8], and the geometric mean titer (GMT) has a high rate of change (GMT ratio: GMR) after vaccination[7-8]. However, the serum antibody titers before and after vaccination and the side effects after the vaccination have not been widely reported, especially in Japan. Therefore, in this study, we analyzed changes in antibody titers from before to after COVID-19 vaccination, factors related to these changes, and health conditions including suspected side effects of vaccination in several thousand medical workers at Kawasaki Gakuen.

MATERIALS AND METHODS

Study design and ethical considerations

Kawasaki Gakuen began a study entitled "A fact-finding survey on retention of serum antibody titer before and after introduction of COVID-19 vaccine, long-term changes in antibody titer, and post-vaccination health conditions" in medical workers at the facility on February 18, 2021 (day of release of jRCT; approval by the ethics committee of Kawasaki Medical School / Kawasaki Medical School Hospital: approval no. 5443; Implementation Plan for jRCT Clinical Study: 1061200057). The goal of this study was to examine changes in antibody titers from before to after COVID-19 vaccination, to identify related background factors, and to record long-term health conditions including side effects after vaccination.

The study reported here was conducted as an interim analysis for the period before vaccination until 4 weeks after the second vaccination. The subjects were 2,304 medical workers who received COVID-19 vaccination as a priority group (2 doses) and agreed to participate in the study and allow use of their information. The study was conducted after obtaining approval from the ethical committee of Kawasaki Medical School / Kawasaki Medical School Hospital (approval no.: 5443) and was performed in accordance with the Helsinki Declaration (Fortaleza revised version, 2013), with Ethical Guidelines for Medical and Biological Research Involving Human Subjects of the Ministry of Education, Culture, Sports, Science

and Technology, Ministry of Health, Labour and Welfare, and Ministry of Economy, Trade and Industry (enforced on June 30, 2021), and with the study protocol. Written informed consent was obtained from the subjects after they were provided with an explanation of the study using leaflets. Data were closely managed using identification codes after deletion of medical record numbers, names, and birth dates to protect privacy.

Antibody titer measurements

Antibody titers were measured using a chemiluminescent enzyme immunoassay (CLEIA), with SARS-CoV-2 antibody S-IgG reagent (Sysmex, Hyogo, Japan) used to determine the serum level of the IgG antibody for SARS-CoV-2 spike protein. Measurements were made using an automated immunoassay system (HISCL-500; Sysmex, Hyogo, Japan) and recorded in Sysmex units (SU)/mL. Titers ≥ 10 SU/mL (criteria of Sysmex Corp.) were considered to be positive, and those ≤ 0.1 SU/mL (lower limit) were recorded as 0.1 SU/mL. Immunogenicity was evaluated using SCR and SPR at 4 weeks after the second vaccination and GMR from before vaccination to 4 weeks after the second vaccination.

Vaccination procedure

SARS-CoV-2 mRNA vaccine (0.3 mL, Comirnaty, Pfizer Inc., NY, USA / BioNTech SE, RP, Germany) was injected intramuscularly twice at an interval of 3 weeks, using a Flomax needle (25 mm, 25G; Nipro, Osaka, Japan), TSK Steriject (25 mm, 25G; TSK Laboratory, Tochigi, Japan), or FN Syringe SS-010F2716 (implantable needle) (16 mm, 27G; Terumo, Tokyo, Japan).

Health status before and after vaccination

Age, sex, vaccination status, and factors that may affect antibody titer in subjects who showed no seroconversion were obtained from medical records. Information on health status before and after vaccination was collected using a self-administered questionnaire. Items collected before vaccination included history of COVID-19 infection, contact with a patient with COVID-19 infection, close contact with persons other than family members in areas under declaration of a state of emergency, past medical history, and history of drug treatment. Subjects were also asked to report symptoms as suspected side effects after the first vaccination, before and after the second vaccination, and for 4 weeks after the second vaccination. Particularly detailed information was collected for one week after each vaccination. Information was requested for pain, swelling, and reddening as side effects at the local injection site, and fever, chill, fatigue, headache, physical pain, and other symptoms (freely described) as systemic side effects.

Statistical analysis

Stat Flex ver. 7 (Artec, Osaka, Japan) was used for statistical analysis. GMT before and after COVID-19 vaccination was compared between two related groups by Wilcoxon signed-ranks test. Comparison of GMT by sex between two independent groups was performed by Mann-Whitney U test. Correlation analysis of age and GMR was conducted using a Spearman rank correlation coefficient test. To identify factors affecting GMR, logistic regression analysis was performed with binary variables grouped based on the median GMT used as objective variables, and sex, age, underlying diseases (hypertension, hyperlipidemia, immunological allergic disease, kidney and urinary tract disease, and malignant tumor), related to changes in antibody titer[4], drug therapy (adrenocortical steroids, and drugs used to treat underlying diseases), and side effects after vaccination used as explanatory variables. In individual tests, $p < 0.05$ was considered to indicate a significant difference.

RESULTS

Background of the subjects before COVID-19 vaccination

The median (95% CI) age of the 2,304 subjects (male: 23.8%, female: 76.2%) was 41 years (23-65 years) (Table 1). Most subjects were in their 20s (26.0%), followed by those in their 40s (25.1%) 50s (19.9%), 30s (19.2%) and 60s or older (9.7%)

Table 1. Background of the subjects (n=2,304) before COVID-19 vaccination

Item	Number (%)
Sex (all subjects)	2304 (100%)
Male	548 (23.8)
Female	1756 (76.2)
Age (years)	41 (23-65)
20-	600 (26.0)
30-	443 (19.2)
40-	579 (25.1)
50-	458 (19.9)
60-	224 (9.7)
Past medical history	762 (33.1)
Hypertension	192 (8.3)
Dyslipidemia	136 (5.9)
Diabetes mellitus	50 (2.2)
Cranial nerve disease	24 (1.0)
Cardiovascular disease	25 (1.1)
Respiratory disease	54 (2.4)
Digestive system disease	58 (2.5)
Hematologic disease	30 (1.3)
Endocrine & metabolic disease	45 (2.0)
Immunological & allergic disease	268 (11.6)
Orthopedic disease	20 (0.9)
Dermatological disease	16 (0.7)
Kidney & urinary tract disease	28 (1.2)
Ophthalmic disease	20 (0.9)
Otorhinolaryngologic disease	20 (0.9)
Psychiatric disease	18 (0.8)
Oral & dental disease	4 (0.2)
Malignant disease	14 (0.6)
Gynecological disease[a]	63 / 1756 (3.6)
Pregnancy[a]	2 / 1756 (0.1)
Medications / Drug History	833 (36.2)
Hypertension	187 (8.1)
Dyslipidemia	143 (6.2)
Diabetes mellitus	46 (2.0)
Cranial nerve disease	21 (0.9)
Cardiovascular disease	12 (0.5)
Respiratory disease	8 (0.4)
Digestive system disease	75 (3.3)
Hematologic disease	35 (1.5)
Endocrine & metabolic disease	50 (2.2)
Immunological & allergic disease	310 (13.5)
Orthopedic disease	35 (1.5)
Dermatological disease	6 (0.3)
Kidney & Urinary tract disease	22 (1.0)
Ophthalmic disease	17 (0.7)
Otorhinolaryngologic disease	10 (0.4)
Psychiatric disease	29 (1.3)
Malignant disease	9 (0.4)
Corticosteroid preparation	59 (2.6)
Gynecological disease[a]	110/1756 (4.8)

Data are shown as n (%), except for age for all subjects, which is shown as median (95% CI)
[a] Female subjects only (n = 1756)

Table 2. Health status of subjects (n = 2261) before COVID-19 vaccination

Item	Number of Subjects	% of Subjects
Past history of COVID-19 infection	1	0.04
Contact with a patient with COVID-19 infection	288	12.7
Less than 5 times	194	8.6
5 times or more	94	4.2
Close contact in areas under state of emergency	294	13.0
Less than 5 times	214	9.5
5 times or more	80	3.5

Table 3. Evaluation of immunogenicity of COVID-19 vaccine (n = 2292 subjects)

Item	Immunogenicity	
Seroconversion rate (SCR)	Positive / Total cases (%)	99.9%
Seroprotection rate (SPR)		99.9%
Geometric mean titer ratio (GMR)	Post-GMT / Pre-GMT ratio (95% CI)	2685.5 (587.8 - 5319.2)

(Table 1). A total of 762 subjects (33.1%) had an underlying disease, with the most common being immunological allergic disease (n = 268, 11.6%), followed by hypertension (n = 192, 8.3%) and hyperlipidemia (n = 136, 5.9%), and 14 (0.6%) had a malignant tumor. Two subjects (0.1%) were pregnant. A total of 833 subjects (36.2%) were receiving drug therapy, with treatment for immunological allergic disease being most common (n = 310, 13.5%), followed by treatment for hypertension (n = 187, 8.1%) and hyperlipidemia (n = 143, 6.2%). Nine subjects (0.4%) were being treated for a malignant tumor and 59 (2.6%) were under treatment with adrenocortical steroids.

The survey of health status before COVID-19 vaccination (Table 2) showed that 1 subject (0.04%) had been infected by COVID-19 and 288 (12.7%) had been in contact with a patient with COVID-19, including 194 (8.6%) and 94 (4.2%) with < 5 and ≥ 5 contacts, respectively. Of 294 subjects (13.0%) with contact with a person other than a family member in areas under a state of emergency, 214 (9.5%) and 80 (3.5%) had < 5 and ≥ 5 contacts, respectively. The classification of contact with a patient with COVID-19 infection and close contact in areas under state of emergency were classified based on the distribution of results.

Evaluation of immunogenicity of the COVID-19 vaccine

The median (95% CI) GMT before COVID-19 vaccination was 0.1 (0.1-0.2) and all subjects had negative results (< 10 SU/mL), including the subject with a history of COVID-19 infection. The median GMT measured 4 weeks after the second COVID-19 vaccination was 274.0 (73.6-534.7) and all subjects had positive results (≥ 10 SU/mL), excluding two who had low IgG before vaccination due to treatment of an underlying disease. Thus, there was a significant difference in GMT before and after vaccination ($p < 0.001$ by Wilcoxon signed-ranks test). The results for SCR (percentage of subjects with serum antibody titer before vaccination is less than 10 SU/ m L and antibody titer after vaccination is 10 SU/ m L or more; 99.9%), SPR (percentage of subjects with serum antibody titer after vaccination is 10 SU/mL or more; 99.9%), and median GMR (2,685.5, 95% CI: 587.8-5,319.2) all indicated high immunogenicity (Table 3).

Safety evaluation of the COVID-19 vaccine

Among side effects at the local injection site, the incidence of pain was highest, followed by enlargement and reddening (Fig. 1). The incidence of pain slightly decreased from 80.31% after

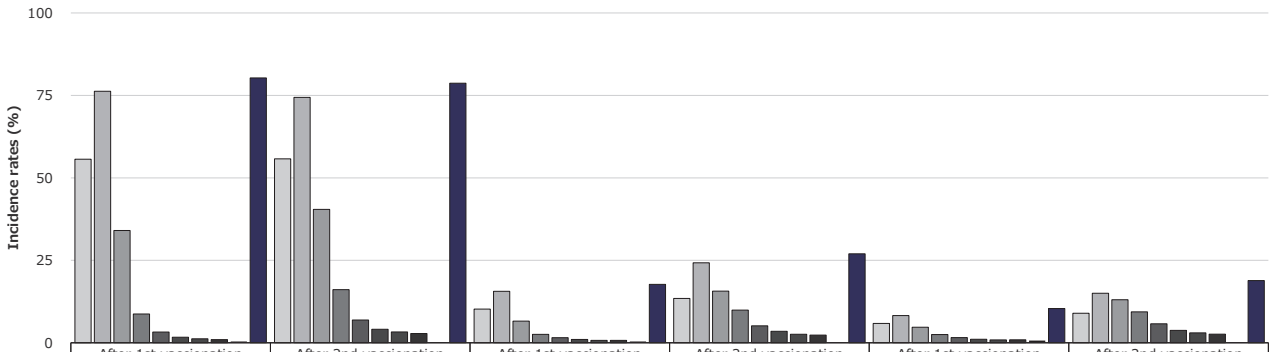
the first vaccination to 78.70% after the second vaccination (Fig. 1). In contrast, the incidences of swelling and reddening increased from 17.72% and 10.36%, respectively, after the first vaccination to 26.97% and 18.87%, respectively, after the second vaccination (Fig. 1).

Among systemic side effects after the first vaccination, the incidence of fatigue was highest (19.88%), followed by headache (15.23%), physical pain (8.17%), chill (5.14%), nausea (4.13%) and fever (1.78%) (Fig. 2). After the second vaccination, these respective incidences were 65.41%, 45.83%, 38.69%, 31.82%, 13.10%, and 39.69%, indicating increases in all items compared to the incidences after the first vaccination (Fig. 2). The incidence of systemic side effects was high on the day after COVID-19 vaccination, except for side effects at the injection site and nausea after the first vaccination (Fig. 1 and 2).

Regarding other side effects, the incidence of skin problems was highest (0.82%) after the first vaccination, followed by upper respiratory tract disorders (0.67%), digestive problems (0.62%), neurological disorder (0.43%), lymph node enlargement (0.38%), disorders of equilibrium (0.19%), oral / dentistry disorders (0.19%), and cardiovascular effects (0.05%) (Fig. 3). These incidences after the second vaccination were 1.57%, 1.05%, 1.43%, 0.57%, 0.81%, 0.48%, 0.33%, and 0.05%, respectively, indicating increases for all items except cardiovascular effects (Fig. 3). In addition, after the second vaccination, disorders of the urinary tract / reproductive tract occurred in 0.19% of the subjects (Fig. 3). There were no serious side effects requiring hospitalization, suggesting the safety of the COVID-19 vaccine.

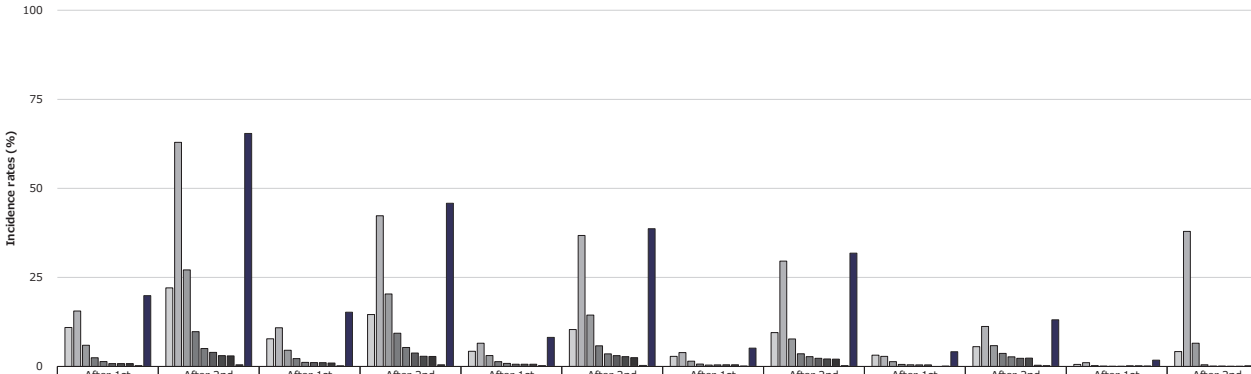
Factors affecting the geometric mean antibody titer ratio (GMR)

Age had a slight negative correlation with GMR ($r = -0.278$) (Fig. 4) and there was a significant difference in median GMR (min-max) after



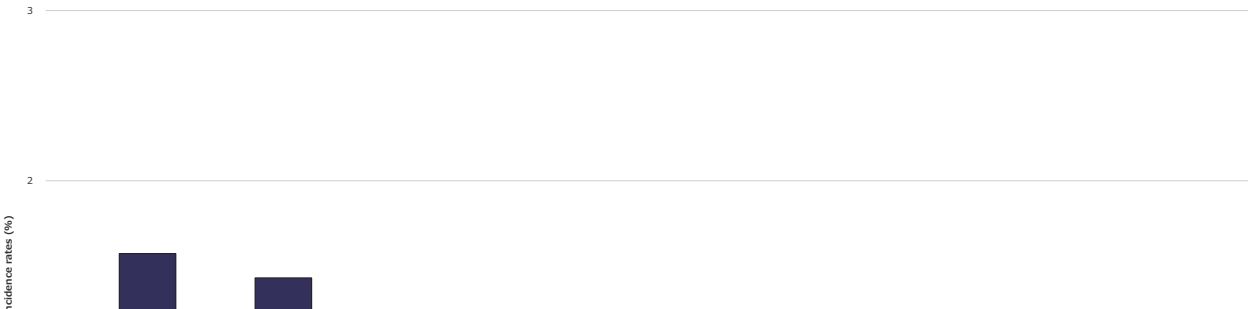
	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation
	Pain		Swelling		Redness	
Day 0	55.67	55.79	10.23	13.43	5.86	8.96
Day 1	76.27	74.42	15.61	24.25	8.26	15.01
Day 2	34.05	40.45	6.58	15.67	4.71	13.05
Day 3	8.74	16.10	2.55	9.91	2.50	9.39
Day 4	3.27	6.91	1.54	5.15	1.59	5.76
Day 5	1.68	4.10	1.01	3.48	1.06	3.76
Day 6	1.20	3.29	0.72	2.57	0.86	3.00
Day 7	0.96	2.81	0.72	2.33	0.91	2.62
Day8-	0.38	0.24	0.38	0.10	0.67	0.14
Total	80.31	78.70	17.72	26.97	10.37	18.87

Fig. 1. Side effects at the local injection site after COVID-19 vaccination.
The incidence of pain was highest, followed by swelling and reddening. All side effects occurred at the highest rates on the day after vaccination. The incidence of pain decreased and those of swelling and reddening increased after the second vaccination, compared to those after the first vaccination.



	Fatigue		Headache		Pain		Chills		Nausea		Fever	
	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation
Day 0	10.95	22.06	7.78	14.58	4.27	10.39	2.83	9.53	3.17	5.57	0.58	4.19
Day 1	15.56	62.93	10.85	42.31	6.53	36.78	3.89	29.59	2.83	11.24	1.06	37.92
Day 2	5.96	27.11	4.56	20.34	3.07	14.44	1.49	7.72	1.34	5.86	0.24	6.53
Day 3	2.45	9.77	2.21	9.34	1.34	5.81	0.67	3.57	0.58	3.67	0.10	0.48
Day 4	1.39	5.05	1.15	5.34	0.86	3.53	0.38	2.76	0.48	2.72	0.05	0.10
Day 5	0.82	3.95	1.10	3.76	0.62	3.05	0.43	2.29	0.43	2.29	0.05	0.10
Day 6	0.82	3.05	1.06	2.86	0.62	2.76	0.48	2.10	0.43	2.33	0.19	0.05
Day 7	0.82	2.95	0.96	2.81	0.62	2.48	0.48	2.05	0.00	0.33	0.19	0.05
Day8-	0.24	0.48	0.19	0.48	0.24	0.29	0.14	0.24	0.10	0.24	0.14	0.19
Total	19.88	65.41	15.23	45.83	8.17	38.69	5.14	31.82	4.13	13.10	1.78	39.69

Fig. 2. Systemic side effects after COVID-19 vaccination.
After the first vaccination, the incidence of fatigue was highest, followed by headache, physical pain, chill, nausea, vomiting and fever. The incidence of all items increased after the second vaccination. The incidence of all side effects except nausea was highest on the day after vaccination.



	Dermal disorder		Digestive disorder		Upper airway disorder		Neural disorder		LN swelling		Equilibrium disorder		Oral & dental disorder		Cardiovascular disorder		Urinary tract & genital disorder	
	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation	After 1st vaccionation	After 2nd vaccionation
	0.82	1.57	0.62	1.43	0.67	1.05	0.43	0.57	0.38	0.81	0.19	0.48	0.19	0.33	0.05	0.05	0.00	0.19

Fig. 3. Other side effects after COVID-19 vaccination.
After the first COVID-19 vaccination, the incidence of skin problems was highest, followed by digestive disorder, upper respiratory tract disorder, neurological disorder, lymph node enlargement, disorder of equilibrium, oral / dentistry disorder, and cardiovascular effects. After the second vaccination, the incidence increased for all events except cardiovascular effects. Urinary tract/reproductive tract disorder also occurred after the second vaccination.

COVID-19 vaccination in male and female subjects (2,280 (75.1-7,124.0) vs. 2,860 (2.0-8,200.0), $p < 0.001$ by Mann-Whitney U test) (Fig. 5). In logistic regression analysis with GMR as the objective variable, and sex, age, underlying diseases related to changes in antibody titer [9], drug therapy, and

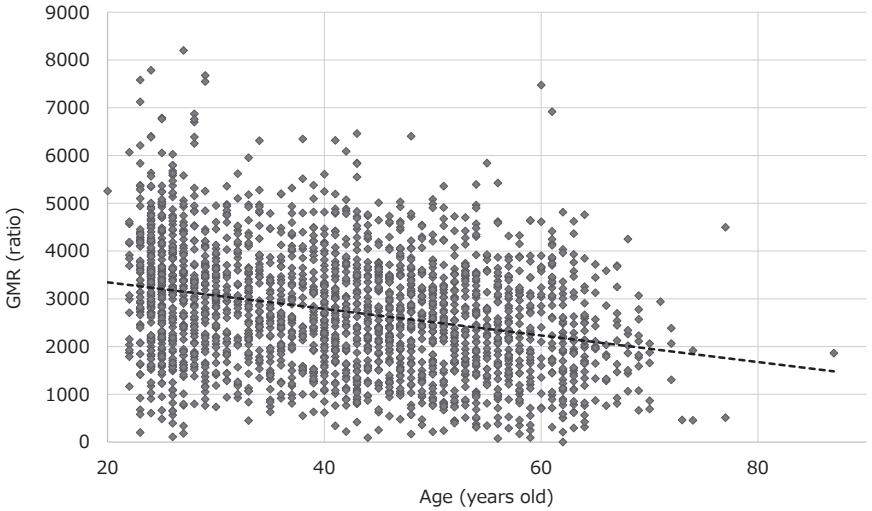
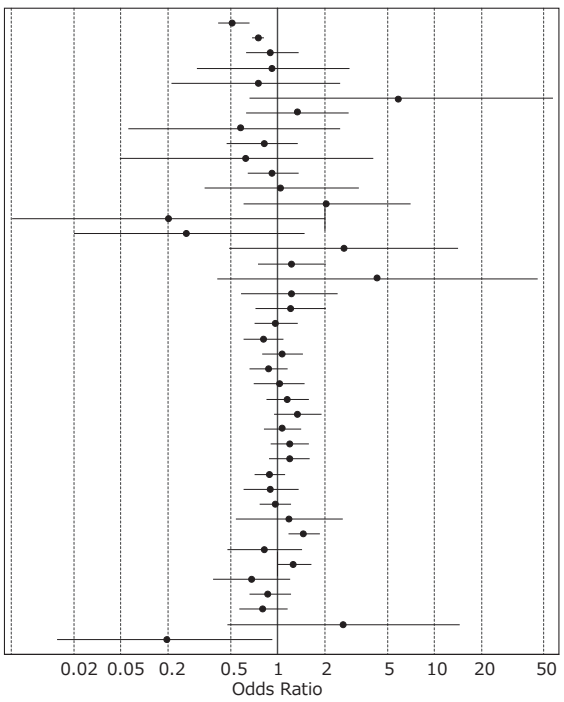


Fig. 4. Correlation analysis for age and GMR.
Age and GMR showed a slight negative correlation in a Spearman rank correlation coefficient test (r = -0.278, p = < 0.001)



Explanatory variable	β	SE(β)	z	p-value	Odds ratio	95%CI
	1.13	0.25				
Sex	-0.65	0.12	-5.41	**<0.01**	0.52	0.41-0.66
Age	-0.29	0.04	-6.97	**<0.01**	0.75	0.69-0.81
Past medical history (PMH)	-0.08	0.20	-0.40	0.69	0.92	0.63-1.37
Hypertension	-0.07	0.57	-0.11	0.91	0.94	0.30-2.88
Dyslipidemia	-0.32	0.63	-0.51	0.61	0.73	0.21-2.52
Diabetes mellitus	1.82	1.14	1.60	0.11	6.15	0.66-56.89
Respiratory disease	0.29	0.39	0.75	0.45	1.34	0.63-2.84
Kidney & urinary tract disease	-0.64	0.79	-0.81	0.42	0.53	0.11-2.50
Immunological & allergic disease	-0.23	0.27	-0.87	0.38	0.79	0.47-1.34
Malignant disease	-0.46	0.95	-0.48	0.63	0.63	0.10-4.07
Medications / Drug History (MDH)	-0.07	0.19	-0.35	0.72	0.94	0.65-1.36
Hypertension	0.06	0.58	0.10	0.92	1.06	0.34-3.30
Dyslipidemia	0.73	0.62	1.16	0.25	2.07	0.61-7.02
Diabetes mellitus	-1.61	1.17	-1.37	0.17	0.20	0.02-1.99
Respiratory disease	-1.30	0.86	-1.51	0.13	0.27	0.05-1.48
Kidney & Urinary tract disease	0.97	0.86	1.13	0.26	2.63	0.49-14.20
Immunological & allergic disease	0.21	0.25	0.81	0.42	1.23	0.75-2.02
Malignant disease	1.46	1.20	1.22	0.22	4.31	0.41-45.28
Corticosteroid preparation	0.17	0.36	0.46	0.65	1.18	0.58-2.40
Local side effect	0.19	0.26	0.73	0.46	1.21	0.72-2.04
Pain (after 1st vaccination)	-0.02	0.16	-0.13	0.89	0.98	0.71-1.35
Pain (after 2nd vaccination)	-0.21	0.15	-1.37	0.17	0.81	0.61-1.09
Swelling (after 1st vaccination)	0.07	0.15	0.46	0.65	1.07	0.80-1.45
Swelling (after 2nd vaccination)	-0.14	0.14	-0.98	0.33	0.87	0.66-1.15
Redness (after 1st vaccination)	0.02	0.19	0.09	0.93	1.02	0.70-1.48
Redness (after 2nd vaccination)	0.15	0.16	0.91	0.36	1.16	0.85-1.58
Systemic side effect	0.30	0.18	1.67	0.10	1.35	0.95-1.91
Fatigue (after 1st vaccination)	0.08	0.14	0.54	0.59	1.08	0.82-1.42
Fatigue (after 2nd vaccination)	0.18	0.14	1.26	0.21	1.20	0.91-1.59
Headache (after 1st vaccination)	0.17	0.15	1.11	0.27	1.19	0.88-1.60
Headache (after 2nd vaccination)	-0.12	0.12	-1.04	0.30	0.89	0.71-1.11
Physical pain (after 1st vaccination)	-0.10	0.21	-0.50	0.62	0.90	0.60-1.35
Physical pain (after 2nd vaccination)	-0.03	0.12	-0.29	0.77	0.97	0.77-1.22
Fever (after 1st vaccination)	0.17	0.40	0.43	0.67	1.19	0.54-2.60
Fever (after 2nd vaccination)	0.39	0.12	3.33	**<0.01**	1.47	1.17-1.85
Chills (after 1st vaccination)	-0.19	0.28	-0.69	0.49	0.83	0.48-1.43
Chills (after 2nd vaccination)	0.25	0.13	1.97	**0.05**	1.28	1.00-1.64
Nausea (after 1st vaccination)	-0.38	0.29	-1.32	0.19	0.68	0.38-1.21
Nausea (after 2nd vaccination)	-0.12	0.16	-0.75	0.45	0.89	0.66-1.21
Other side effect	-0.21	0.18	-1.16	0.25	0.81	0.57-1.16
LN swelling (after 1st vaccination)	0.96	0.87	1.11	0.27	2.62	0.48-14.48
LN swelling (after 2nd vaccination)	-1.66	0.81	-2.05	**0.04**	0.19	0.04-0.93

Fig. 5. Factors affecting change of GMR.
Logistic regression analysis with GMR as the objective variable, and sex, age, underlying diseases related to changes in antibody titer, drug therapy, and side effects after vaccination used as explanatory variables, showed sex, age, fever, chill and lymph node enlargement after the second vaccination were significantly associated with GMR. Subjects were divided into two groups based on a median of 2,685.5, n = 2,037, AIC = 2,680.86, AUC = 0.68. β: partial regression coefficient, SE (β): standard error (β), z = β / SE (β).

side effects after vaccination used as explanatory variables, sex (odds ratio (OR) 0.12, 95% CI 0.41-0.66), age (OR 0.75, 95% CI 0.69-0.81), fever (OR 1.47, 95% CI 1.17-1.85), chill (OR 1.28, 95% CI

1.00-1.64) and lymph node enlargement (OR 0.19, 95% CI 0.04-0.93) after the second vaccination were significantly associated with GMR ($p < 0.05$) (Fig. 5).

DISCUSSION

Summary and novelty of the study

This study shows the high immunogenicity of the COVID-19 vaccine based on serum antibody titers and supports the safety of the vaccine based on a health survey. The results provide new evidence for the actual effects of the vaccine, and suggest that sex, age, fever, chill, and lymph node enlargement after the second vaccination are factors affecting GMR.

Mechanism of action of the COVID-19 mRNA vaccine

In patients with COVID-19 infection, the spike protein uses angiotensin-converting enzyme 2 (ACE2) on cells as a receptor, and transmembrane protease, serine 2 (TMPRSS2) plays an auxiliary role in invasion of cells by viral particles[10]. These particles release RNA in the host cells, and parts of the RNA are then translated into protein in the endoplasmic reticulum. The translated protein forms a replication complex to produce more RNA, and the RNA and protein assemble as new viral particles with use of the Golgi apparatus, with subsequent release of new viruses[11-12].

The COVID-19 mRNA vaccine has mRNA as an active element that encodes spike protein. When injected intramuscularly, the vaccine enables protein to be produced with mRNA used as a template in immunocompetent cells, such as muscle cells and dendritic cells. Antigen presentation of parts of the produced protein then induces an immune response[13]. Since mRNA is readily digested by ribonucleases in vivo, lipid nanoparticles (LNPs) are used as a drug delivery system (DDS) to deliver mRNA and enhance immunogenicity through stimulation of natural immunity by LNPs as an adjuvant[14]. A neutralization antibody for vaccine-induced spike protein has been found to inhibit cellular invasion of COVID virus and induce humoral immunity by antibodies and cell immunity by cytotoxic T-lymphocytes[15]. Secretor IgA on airway mucosa also has an important role in preventing infection, and has been detected in saliva at rates of 54.7% and 84.6% using Comirnaty and Spikevax (Moderna Inc., Cambridge, MA, USA / Takeda Pharma Co. Ltd., Tokyo, Japan), respectively[16]. These rates suggest that COVID-19 mRNA vaccine has a high level of immunity induction.

Safety of the COVID-19 mRNA vaccine

Previous studies of mRNA vaccines have found incidences of pain at the local injection site of 70% to < 90%[1-3]. A moderate or higher level of pain that might interfere with daily activities occurred at rates of about 30% and 15% after the first and second vaccinations, respectively, and severe pain that affected daily life had rates of 0.7% and 0.9% after the respective vaccinations[1]. In the current study, pain was also the most common local side effect, followed by swelling and reddening. These side effects were mostly observed on the day after vaccination. The incidence of pain decreased and those of swelling and reddening increased after the second vaccination, compared to the first vaccination.

mRNA vaccination also causes systemic side effects, such as fever, headache, fatigue, chill, nausea / vomiting, and muscle pain[2, 17]. In studies of Comirnaty, the incidence of fever (≥ 38℃) was low after the first vaccination, but increased to 8-17% after the second vaccination, and with a tendency for a higher incidence in younger subjects[2, 17]. A higher incidence of other systemic side effects also occurred after the second vaccination[2, 17]. Spikevax caused similar incidences of side effects to those

with Comirnaty[8, 18]. In the current study, systemic side effects of fever, headache, fatigue, chill, nausea / vomiting and muscle pain were similarly found. The incidence of fever (commonly defined in Japan as a temperature ≥ 37.5℃) increased from 1.78% after the first vaccination to 39.69% after the second vaccination. Skin, digestive tract, upper respiratory tract, and neurological disorders, lymph node enlargement, disorders of equilibrium, oral and cardiovascular effects, and urinary tract / reproductive tract disorders were also observed.

Serious side effects have been found at a rate of 0.6% in clinical studies with Comirnaty[1] and Spikevax[2]. Anaphylactic shock occurred at incidences of 11.1%[19] and 2.5%[20] among 1 million vaccinations with Comirnaty and Spikevax, respectively, and a meta-analysis showed incidences of anaphylactic shock among 1 million vaccinations of 8.0% and 2.8% with the respective vaccines[21]. Female subjects and subjects with a history of shock have been found to account for 94.5% and 38.7% of cases with anaphylactic shock, respectively, and 77.4% and 87.1% of cases occurred within 15 and 30 minutes after vaccination, respectively[19, 20]. Polyethylene glycol (PEG) included in LNP may be a causative agent for anaphylactic shock due to an immediate hypersensitivity reaction via IgE[22]. Since PEG is widely used in drugs and cosmetics, subjects with a history of allergy for such products are a particular concern. Post-vaccination development of myocarditis has also been reported, with a higher incidence in males aged 10-20 after the second vaccination. The rates of post-vaccination myocarditis reported by the Vaccine Adverse Event Reporting System (VAERS) in the US were 40.6 and 2.4 cases in 1 million second vaccinations in males aged 12-29 and ≥ 30 years, respectively, and most cases were mild[23]. In the current study, there were no serious side effects requiring hospitalization.

Side effects of vaccines are defined as health disadvantages induced or suspected to be induced by the vaccine. There are no vaccines without side effects, and local side effects develop at a high rate and systemic symptoms at a certain rate, but many are transient events. In addition, since mRNA is degraded immediately and thus, does not remain in cells for a long period of time and is not integrated into chromosomes, its safety is relatively high. However, serious side effects, such as anaphylactic shock, may develop immediately after vaccination and myocarditis may develop occasionally. Persons who notice a subjective symptom after vaccination should visit a hospital as soon as possible, and medical staff should treat these patients with consideration of the possibility of side effects of the vaccine.

Efficacy of the COVID-19 mRNA vaccine

The efficacy of COVID-19 vaccine has been evaluated in clinical studies and in real world settings, with immunogenicity used as an index. In phase III clinical studies, the efficacy rates of mRNA vaccine to prevent infection were 95.0% in subjects aged ≥ 16 years who received Comirnaty[1] and 94.1% in those aged ≥ 18 years who received Spikevax[2]. Efficacy in real world settings is also evaluated based on prevention of infection, hospitalization, and high severity. In a large-scale comparative study with Comirnaty in Israel, development of infection on day 7 or later after the second vaccination, hospitalization, and severe infection were decreased by 94%, 87%, and 92%, respectively, in the vaccine group compared to the no-vaccine group[4]. The vaccine also prevented infection by 92% based on regular PCR tests conducted on day 7 or later after the second vaccination[4]. Reports in the US suggest that Comirnaty and Spikevax decreased the infection rate in medical workers by 94% on day 7 or later[5] and in the general population by 90% on day 14 or later[6] after the second vaccination.

Vaccine-induced immunity includes humoral

immunity and cellular immunity. Humoral immunity is generally used for evaluation of immunogenicity because it can be measured easily. The immunogenicity of COVID-19 vaccine is evaluated based on the ratio of vaccine recipients with a serum antibody titer that reaches a level to prevent infection. The evaluation indexes include GMR, SCR, and SPR. In an overseas phase I clinical study with Comirnaty (30 μg), GMTs measured on day 14 after the second vaccination were 8,147 and 6,014 U/mL in subjects aged 18-55 and 65-85 years, respectively, and the GMRs compared to before vaccination were 13,578.3 and 10,523.3, respectively[17]. In an overseas phase I / II clinical study with Spikevax (100 μg), GMTs measured on day 14 after the second vaccination were 1,909 and 1,686 U/mL in subjects aged 18-55 and ≥ 55 years, respectively, and the GMRs compared to before vaccination were 41.5 and 36.7, respectively[8, 18]. The seroconversion rate of subjects aged ≥ 18 years after the second COVID-19 vaccination was 100%[8, 18].

The efficacy and safety of drugs including vaccines are affected by differences in race, which makes it important to conduct a study in Japan. Our evaluation of the immunogenicity of COVID-19 vaccine in 2,304 medical workers aged ≥ 20 years gave results for median GMR, SCR, and SPR of 2,685.5, 99.9%, and 99.9%, respectively, at 4 weeks after the second vaccination. This confirms the high immunogenicity of the COVID-19 vaccine in Japanese subjects. The results also suggested that sex, age, systemic side effects, fever, and lymph node enlargement after the second vaccination have significant effects on GMR. Side effects occur at a certain rate, but systemic side effects such as fever and lymph node enlargement might still be useful indexes for the effectiveness of immunization with the vaccine.

However, our study has four limitations. First, the study includes the subjects all being adult medical workers at our facility, and a study with a wider range of subjects is required.

Second, the study examined only changes in serum antibody titers, the evaluation of preventive effect of the COVID-19 mRNA vaccine (e.g., infection prevention) is not sufficient, and a study with further follow-up is required. Third, there are differences in measurement kits, methods, and equipment for each report, and not all reports can be compared equally. Finally, the observation period was only 4 weeks after the second vaccination, and thus, the persistency of immunity was not examined. Further analyses of longer-term data will be reported in the future.

CONCLUSIONS

Serum antibody titers showed the high immunogenicity of the COVID-19 vaccine, and a health condition survey suggested acceptable safety of the vaccine. Systemic side effects such as fever and lymph node enlargement may be useful as indexes of vaccination efficacy.

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AUTHORSHIP STATEMENT

S.O., T.Nakan., T.T., T.K., E.K., T.O. and A.N. designed the study. T.H. and M.A. contributed to sample collection. R.U., Y.K., M.T and K.T. contributed to sample quantification. T.Nakat. and K.H. contributed to data collection. S.O. and E.K.

analyzed the data. S.O. and T.Nakan. wrote the manuscript. All authors reviewed and approved the manuscript.

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CONFLICT OF INTEREST

None.

REFERENCES

1）Polack FP, Thomas SJ, Kitchin N, *et al.*: Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. N Engl J Med 2020; 383: 2603-2615. doi: 10.1056/NEJMoa2034577.

2）Baden LR, El Sahly HM, Essink B, *et al.*: Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med 2021; 384: 403-416. doi: 10.1056/NEJMoa2035389.

3）Ramasamy MN, Minassian AM, Ewer KJ, *et al.*: Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet 2021; 396: 1979-1993. doi: 10.1016/s0140-6736(20)32466-1.

4）Dagan N, Barda N, Kepten E, *et al.*: BNT162b2 mRNA COVID-19 vaccine in a nationwide mass vaccination setting. N Engl J Med 2021; 384: 1412-1423. doi: 10.1056/NEJMoa2101765.

5）CDC COVID-19 Response Team; Food and Drug Administration. Largest CDC COVID-19 Vaccine Effectiveness Study in Health Workers Shows mRNA Vaccines 94% Effective. ttps://www.cdc.gov/media/releases/2021/p0514-covid-19-vaccine-effectiveness.html. Accessed Dec 7, 2021.

6）CDC COVID-19 Response Team; Food and Drug Administration. CDC Real-World Study Confirms Protection Benefits of mRNA COVID-19 Vaccines. https://www.cdc.gov/media/ releases/2021/p0329-COVID-19-Vaccines.html. Accessed Dec 7, 2021.

7）Miyakawa K, Jeremiah SS, Ohtake N, *et al.*: Rapid quantitative screening assay for SARS-CoV-2 neutralizing antibodies using HiBiT-tagged virus-like particles. J Mol Cell Biol 2020; 12: 987-990. doi: 10.1093/jmcb/mjaa047.

8）Ali K, Berman G, Zhou H, *et al.*: Evaluation of mRNA-1273 SARS-CoV-2 vaccine in adolescents. N Engl J Med 2021; 385: 2241-2251. doi: 10.1056/NEJMoa2109522

9）Vanshylla K, Di Cristanziano V, Kleipass F, *et al.*: Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. Cell Host Microbe 2021; 29: 917-929. e4. doi: 10.1016/j.chom.2021.04.015.

10）Hoffmann M, Kleine-Weber H, Schroeder S, *et al.*: SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020; 181: 271-280. doi: 10.1016/j.cell.2020.02.052.

11）Song Z, Xu Y, Bao L, *et al.*: From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 2019; 11: 59. doi: 10.3390/v11010059.

12）Jiang S, Lu L, Liu Q, *et al.*: Receptor-binding domains of spike proteins of emerging or re-emerging viruses as targets for development of antiviral vaccines. Emerg Microbes Infect 2012: 1; e13. doi: 10.1038/emi.2012.1.

13）Versteeg L, Almutairi MM, Hotez PJ, *et al.*: Enlisting the mRNA vaccine platform to combat parasitic infections. Vaccines (Basel) 2019; 7: 122. doi: 10.3390/vaccines7040122.

14）Schoenmaker L, Witzigmann D, Kulkarni JA, *et al.*: mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. Int J Pharm 2021; 601: 120586. doi: 10.1016/j.ijpharm.2021.120586.

15）Shirai S, Kawai A, Shibuya M, *et al.*: Lipid nanoparticle acts as a potential adjuvant for influenza split vaccine without inducing inflammatory responses. Vaccines (Basel) 2020; 8: 433. doi: 10.3390/vaccines8030433.

16）Ketas TJ, Chaturbhuj D, Portillo VMC, *et al.*: Antibody responses to SARS-CoV-2 mRNA vaccines are detectable in saliva. Pathog Immun 2021; 6: 116-134. doi: 10.20411/pai.v6i1.441.

17）Walsh EE, Frenck Jr RW, Falsey AR, *et al.*: Safety and immunogenicity of two RNA-based COVID-19 vaccine candidates. N Eng J Med 2020; 383 :2439-2450. doi: 10.1056/NEJMoa2027906.

18) Chu L, McPhee R, Huang W, *et al.*: A preliminary report of a randomized controlled phase 2 trial of the safety and immunogenicity of mRNA-1273 SARS-CoV-2 vaccine. Vaccine 2021; 39: 2791-2799. doi: 10.1016/j.vaccine.2021.02.007.

19) CDC COVID-19 Response Team; Food and Drug Administration. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Pfizer-BioNTech COVID-19 Vaccine - United States, December 14-23, 2020. MMWR Morb Mortal Wkly Rep 2021; 70: 46-51. doi: 10.15585/mmwr.mm7002e1.

20) CDC COVID-19 Response Team; Food and Drug Administration. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Moderna COVID-19 Vaccine - United States, December 21, 2020-January 10, 2021. MMWR Morb Mortal Wkly Rep 2021; 70: 125-129. doi: 10.15585/mmwr.mm7004e1.

21) Alhumaid S, Al Mutair A, Al Alawi Z, *et al.*: Anaphylactic and nonanaphylactic reactions to SARS-CoV-2 vaccines: a systematic review and meta-analysis. Allergy Asthma Clin Immunol 2021; 17: 109. doi: 10.1186/s13223-021-00613-7.

22) Turner PJ, Ansotegui IJ, Campbell DE, *et al.*: COVID-19 vaccine-associated anaphylaxis: A statement of the World Allergy Organization Anaphylaxis Committee. World Allergy Organ J 2021; 14: 100517. doi: 10.1016/j.waojou.2021.100517.

23) Gargano JW, Wallace M, Hadler SC, *et al.*: Use of mRNA COVID-19 vaccine after reports of myocarditis among vaccine recipients: Update from the Advisory Committee on Immunization Practices - United States, June 2021. MMWR Morb Mortal Wkly Rep 2021;70:977-982. doi: 10.15585/mmwr.mm7027e2.