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ORIGINAL ARTICLE

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Prevalence of hepatitis B and C, and their linkage to care among drug abusers attending psychiatric hospital in Hiroshima, Japan

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Abstract: Towards the WHO goal for hepatitis elimination, understanding the prevalence and management of hepatitis B and C viruses (HBV, HCV) among drug abusers is crucial. However, in Japan, where drug abuse is less prevalent than in other countries, there is a dearth of epidemiological studies on this topic. This study aimed to fill this gap by investigating virus prevalence and the testing and treatment landscape for drug abusers in Japan. We conducted a cross-sectional sero-epidemiological study at a psychiatric hospital in Hiroshima where approaching drug abusers was feasible. Blood samples and questionnaire on HBV/HCV testing and treatment were collected from drug abusers (n = 35, 85.7% male, mean age 55.4 years) and control group (n = 45, 71.1%, 48.2 years). Prevalence of HCV-Ab and HCV RNA in drug abusers was 60.0% (95% CI: 43.8–76.2%) and 28.6% (13.6–43.5%), respectively, which was significantly higher than in the control group (2.2%, 0.0%, respectively). All HCV-Ab positive drug abusers had undergone prior hepatitis virus testing, but only 42.9% of those eligible for HCV treatment were connected to it. For HBV, while prevalence of HBsAg was similar between groups (2.9% *vs*. 2.2%), prevalence of HBc-Ab was higher in drug abusers (34.3% *vs.* 17.8%), indicating a greater likelihood of exposure to HBV infection. In conclusion, HCV prevalence among drug abusers in psychiatric care is notably high. Although testing is accessible, a recognized challenge is the insufficient connection to treatment. Enhancing collaboration between psychiatric hospitals and hepatologists is crucial. Disregarding this issue is not an option for hepatitis elimination.

***Keywords*:** HBV, HCV, sero-epidemiology, drug abusers, micro-elimination, Japan

Introduction

Data from the World Health Organization (WHO) indicate that as of 2019, there were 296 million people globally living with chronic hepatitis B virus (HBV) infection and 58 million with chronic hepatitis C virus (HCV) infection, with 1.5 million new infections each year. HBV and HCV infections can lead to liver cirrhosis and hepatocellular carcinoma, resulting in over 820,000 deaths for HBV and 290,000 deaths for HCV (*1,2*).

In Japan, various initiatives have been implemented for the prevention and management of HBV and HCV infections (*3,4*). These include the screening system for blood donors for HBV and HCV, with hepatitis B surface antigen (HbsAg) screening in place since 1972 and HCV antibody (HCV-Ab) screening since 1989. Additionally, a national project has been established since 1986 to prevent mother-to-child transmission of HBV. Since 2002, there has been a nationwide screening program targeting residents aged 40 years and over for HBV and HCV. Furthermore, since 2007, regional core hospitals for the treatment of liver diseases have been established in every prefecture. This network aims to enhance the capacity for managing liver-related conditions across the country. Additionally, since 2008, a medical expense subsidy system has been in operation to support the antiviral treatment of individuals infected with HBV or HCV. The Basic Act on Hepatitis Measures was enacted in 2009, providing a legal framework for both the national government and local authorities to implement countermeasures based on this legislation. As a result of these measures, the number of individuals with sustained infections of HBV and HCV decreased from a range of 3.01 to 3.66 million people in the year 2000 to a range of 1.91 to 2.49 million people in 2015 (*5*). In Japan, the prevalence of HBsAg and HCV-Ab is controlled at 0.37% and 0.28%, respectively, indicating a globally low level of infection (*5,6*).

WHO has established assessment criteria related to incidence, mortality, and health service coverage associated with prevention and treatment for the goal of eliminating Viral Hepatitis by 2030. For HBV, as there is currently no curative treatment to eliminate the virus, achieving the goal by 2030 is anticipated to be challenging in all countries, including Japan and worldwide (*7*). Regarding HCV, it is anticipated that Japan will achieve elimination by 2030 (*7*). However, to achieve this target, special attention should be given to the detection and effective treatment of high-risk populations such as drug abusers, including people who inject drugs (PWID), people living with HIV (PLHIV), prisoners and the homeless (*8*).

As for PWID, approximately 15.6 million PWIDs worldwide are between 15 and 64 years of age (*9*), and more than half of them (52.3%) are HCV-Ab positive and 9.0% are HBs-Ag positive, according to a previous systematic review (*9*). In the United States, 2.6% of adults report a history of injection drug use, with over 50% testing positive for HCV-Ab (*10*). In most countries, the risk of HCV infection among PWID is a major concern due to insufficient awareness and widespread unsafe injection practices (*11*).

Compared to the global situation, Japan has a significantly lower number of PWID. For example, while the estimated PWID population in the United States was over 3.7 million in 2018 (*12*), Japan had 0.5 million in 2011 (*13*). Consequently, little attention has been paid to this group, and their infection and treatment status remain unknown. PWID in Japan are either in prison or hidden in society, making it difficult to conduct a survey among them.

This study conducted within a psychiatric hospital setting offers a feasible approach to reach this hidden population. It provides an opportunity to collect data and investigate a sero-epidemiological investigation among psychiatric patients with a history of drug abuse at a psychiatric hospital, with a control group of patients without a history of drug abuse. Our goal was to clarify the current sero-epidemiological status of hepatitis viruses among them and to assess the linkage to care conditions of HCV treatment-eligible patients with a history of drug abuse.

Patients and Methods

We conducted a cross-sectional sero-epidemiological study from November 2021 to March 2022 to estimate the prevalence of HBV and HCV infections among patients at a psychiatric hospital in Hiroshima City, including those with and without a history of drug abuse. Additionally, we explored their history of treatment for HBV or HCV using a self-administered questionnaire.

Ethics approval and patient informed consent

This study was approved by the Hiroshima University Epidemiological Ethics Review Committee and the Senogawa Hospital Ethics Committee (E-2634, R03). Informed consent was obtained from all participants before any study procedure was conducted. All study activities were performed following the Declaration of Helsinki and relevant guidelines and regulations in Japan.

Study population

For this study, we recruited psychiatric patients with or without a history of drug abuse. The control group consisted of patients without a history of drug abuse. Participants were included if they expressed a desire to participate and provided written consent. Exclusions comprised individuals with an unknown history of drug abuse or those with severe mental illness lacking the capacity to provide consent. We used a voluntary convenient sampling method and recruited a total of 80 psychiatric patients, including 35 with a history of drug abuse and 45 without.

Samples collection and sero-molecular analysis of HBV and HCV

A questionnaire was administered to the subjects using a survey form consistently employed by the Ministry of Health, Labour and Welfare research group for general populations (*14*). This comprehensive questionnaire covered aspects such as past hepatitis virus tests and treatment history in the event of a positive result. Notably, a section on drug abuse history was incorporated into the questionnaire specifically for this study. The survey questionnaire was self-administered by clinically stable psychiatric patients; however, individuals with visual impairments or handwriting difficulties due to tremors, for instance, had a physician read the questionnaire, and the individual verbally responded while the physician recorded the answers.

We collected 10 ml of intravenous blood samples from each participant. All blood samples collected were tested for hepatitis B, C, and liver function tests (AST and ALT). HCV-Ab (Lumipulse II Ortho HCV antibody, Fujirebio Inc, Tokyo, Japan) was detected by a chemiluminescent immunoassay (CLIA) with a signal to cutoff ratio of 1. Hepatitis B serological tests were done using Lumipulse Presto HBsAg-HQ (Fujirebio Inc, Tokyo, Japan) for HBsAg with cutoff at 0.005 IU/mL, Lumipulse Presto HBs-Ab-III (Fujirebio Inc, Tokyo, Japan) for HBs-Ab with cutoff at 10.0mIU/mL, and Lumipulse Presto HBc-Ab-III (Fujirebio Inc, Tokyo, Japan) for HBc-Ab with cutoff at 1.0 cutoff index.

All serum samples positive for HBsAg or HCV-Ab were underwent further molecular analysis. The viral titer was determined for HBV DNA and HCV RNA using Taqman Fast advanced master mix (Thermo Fisher

Scientific, MA, USA) and Fast 1 Step Mix (Thermo Fisher Scientific, MA, USA) on Applied Biosystems StepOne (Thermo Fisher Scientific, MA, USA). The positive PCR samples were then employed for amplification of surface polymerase gene for HBV and core gene for HCV by first and second rounds of nested PCR followed by Sanger sequencing as per previous reports (*15-17*). The genotypes of both HBV and HCV were determined after the phylogenetic tree analysis of sample genomes and retrieved reference genomes from GeneBank was constructed by the Neighbor-joining method with Molecular Evolutionary Genetic Analysis 10 (MEGA-X, Pennsylvania State University, PA, USA).

The test results were communicated to individual patients by a hospital doctor and a record card of the hepatitis virus test was issued to all study participants, regardless of their results. Patients who tested positive for the hepatitis virus were referred to the Hiroshima Prefecture Liver Disease follow-up system and advised to undergo medical examinations.

Data analysis

Statistical analyses were performed by using JMP software (SAS Institute, California, USA). Chi-square (X2) test was used, and a p-value < 0.05 was considered statistically significant.

Results

Participant characteristics

The mean age of psychiatric patients with a history of drug abuse (n = 35) was 55.4 ± 11.0 years, and 85.7% were male (Table 1). One person had an occupation related to medical and nursing care. The mean age of psychiatric patients without a history of drug abuse (n = 45) was 48.2 ± 14.9 years, and 71.1% were male. Among them, 18 (40.0%) had occupations related to medical and nursing care.

Status of HCV infections

In this study, 22 out of 80 psychiatric patients were found to be positive for HCV-Ab, with 21 having a history of drug abuse and one without. We showed their individual characteristics in Table 2. All patients had undergone hepatitis virus testing prior to their involvement in this study. Four of the patients were unaware of their past testing status but were considered to have been tested based on their history of surgery, childbirth, and blood donation. Among the 21 HCV-Ab positive psychiatric patients with a history of drug abuse, 7 (33.3%) were HCV RNA negative without any treatment history (infection eliminated naturally), 4 (19.0%) were HCV RNA negative with a treatment history (successfully treated cases), 8 (38.1%) were HCV RNA positive without a treatment history (cases lacking necessary treatment), and 2 (9.5%) were HCV RNA-positive with a treatment history (unsuccessfully treated cases, Case 4, and Case 10 in Table 2). Among the 14 patients eligible for HCV treatment, 6 (42.9%) were linked to the care, and 8 (57.1%) were not linked to the care (Figure 1).

In contrast, among psychiatric patients without a history of drug abuse, only one case tested positive for HCV-Ab. This patient had a history of HCV treatment, and HCV RNA was not detected (Case 22 in Table 2).

The prevalence of HCV-Ab and HCV RNA among psychiatric patients with a history of drug abuse was 60.0% (21/35, 95% CI: 43.8–76.2%) and 28.6% (10/35, 95% CI: 13.6–43.5%), respectively, which was significantly higher than in psychiatric patients without a history of drug abuse (HCV-Ab 2.2%, 95% CI: 0–6.5%, p < 0.0001; HCV RNA 0.0%, 95% CI: 0–8.2%, p = 0.0001, Figure 2).

Status of HCV RNA-positive patients

In this study, we identified 10 patients who were HCV RNA-positive, all of whom had a history of drug abuse. Among these patients, 7 were male and 3 were female, and their ages ranged from 40 to 78 years. Moreover, 6

Table 1. Age and sex distribution of psychiatric patients stratified by drug abuse history

Characteristics	Total (n = 80)		Psychiatric patients with history of drug abuse (n = 35)		Psychiatric patients with no history drug abuse (n = 45)	
	n	%	*n*	%	*n*	%
With history of drug abuse	35	43.8%				
With no history of drug abuse	45	56.3%				
Gender						
Male	62	77.5%	30	85.7%	32	71.1%
Female	18	22.5%	5	14.3%	13	28.9%
Age						
Median (IQR)	50.5 (19.0)		54 (25.8)		47 (21.0)	
Mean age (SD)	51.4 (13.8)		55.4 (11.0)		48.2 (14.9)	

IQR, interquartile range; SD, standard deviation.

Table 2. Characteristics of 22 HCV-Ab positive psychiatric patients

Basic information				Testing and treatment history for HCV				HCV					HBV		
Case	History of drug abuse	Age	Sex	Testing	Detailed examination	HCV treatment	Hope for HCV treatment	HCV-Ab titer (C.O.I.)	RT-qPCR (Ct)	Viral Load (copies/mL)	Nested PCR	HCV Genotype	HBs-Ag	HBs-Ab	HBc-Ab
1	Yes	43	M	Yes (Before surgery, unrecognized)	No	No	Hope	91.0	23.36	1,008,743	(+)	1b	(-)	(-)	(-)
2	Yes	78	M	Yes (Hospital)	No	No	Hope	78.4	30.01	9,547	(+)	1b	(+)	(-)	(+)
3	Yes	40	F	Yes, (Before surgery and childbirth, unrecognized)	No	No	Hope	88.0	22.47	4,355,578	(+)	2b	(-)	(-)	(-)
4	Yes	54	M	Yes (Hospital)	Yes	Yes	Hope	100.0	31.89	2,345	(+)	2b	(-)	(-)	(-)
5	Yes	49	M	Yes (Hospital)	No	No	Hope	62.8	29.45	45,998	(+)	2a	(-)	(-)	(-)
6	Yes	51	F	Yes (Hospital)	No	No	Don't know	68.5	32.01	1,267	(+)	2a	(-)	(+)	(+)
7	Yes	67	M	Yes (Hospital)	No	No	Hope	75.1	28.75	23,567	(+)	1b	(-)	(+)	(+)
8	Yes	52	M	Yes (Hospital)	No	No	Don't know	88.7	21.11	40,385,056	(+)	1b	(-)	(-)	(-)
9	Yes	53	F	Yes (Hospital)	No	No	Hope	26.9	25.35	651,379	(+)	2a	(-)	(+)	(+)
10	Yes	60	M	Yes (Hospital)	Yes	Yes	Hope	5.3	29.26	14,322	(+)	1b	(-)	(+)	(+)
11	Yes	60	M	Yes (Hospital)	No	No	Hope	55.9	-	Undetermined	(-)	-	(-)	(-)	(+)
12	Yes	53	M	Yes (Hospital)	No	No	Don't know	80.5	-	Undetermined	(-)	-	(-)	(-)	(-)
13	Yes	45	M	Yes (Hospital)	Yes	Yes	Hope	21.2	-	Undetermined	(-)	-	(-)	(-)	(-)
14	Yes	58	M	Yes (Hospital)	Yes	Yes	Hope	38.8	-	Undetermined	(-)	-	(-)	(-)	(-)
15	Yes	61	M	Yes (No answer)	Yes	Yes	Hope	11.8	-	Undetermined	(-)	-	(-)	(-)	(-)
16	Yes	50	M	Yes (Hospital)	No	No	Hope	17.0	-	Undetermined	(-)	-	(-)	(-)	(-)
17	Yes	60	M	Yes (Hospital)	No	No	Hope	5.0	-	Undetermined	(-)	-	(-)	(-)	(+)
18	Yes	73	M	Yes (Hospital)	No	No	Hope	5.5	-	Undetermined	(-)	-	(-)	(+)	(+)
19	Yes	61	M	Yes (Hospital)	Yes	Yes	Hope	3.2	-	Undetermined	(-)	-	(-)	(-)	(-)
20	Yes	65	F	Yes (Before surgery, unrecognized)	No	No	Hope	18.2	-	Undetermined	(-)	-	(-)	(+)	(+)
21	Yes	71	M	Yes (Before surgery, blood donation, unrecognized)	No	No	Don't want	45.2	-	Undetermined	(-)	-	(-)	(-)	(-)
22	No	42	M	Yes (Hospital)	Yes	Yes	Hope	47.8	-	Undetermined	(-)	-	(-)	(-)	(-)

HBV, hepatitis B virus; HCV, hepatitis C virus; HCV-Ab, hepatitis C virus antibody; C.O.I, cut off index; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; Ct, cycle threshold; HBs-Ag, hepatitis B surface antigen; HBs-Ab, hepatitis B surface antibody; HBc-Ab, hepatitis B core antibody; LFT, liver function test; AST, aspartate transferase; ALT, alanine transferase; U/L, unit per liter. [†]HCV screening for donated blood has been introduced since 1989 in Japan.

Table 2. Characteristics of 22 HCV-Ab positive psychiatric patients (continued)

Basic information				LFT		Risk factors for infection					Awareness of infection risk by sharing needle		
Case	History of drug abuse	Age	Sex	AST (U/L)	ALT (U/L)	Frequency of sharing syringes or needles with others	History of blood transfusions before 1989[†]	Tattoos	Piercing	Medical staff	HBV	HCV	HIV
1	Yes	43	M	42	45	1–49 times	No	Yes	Yes	No	Know	Know	Know
2	Yes	78	M	26	18	> 100 times	Yes	No	No	No	Don't know	Don't know	Don't know
3	Yes	40	F	37	37	1–49 times	No	No	Yes	No	Know	Know	Know
4	Yes	54	M	39	38	1–49 times	No	No	No	No	Don't know	Know	Don't know
5	Yes	49	M	47	56	1–49 times	No	Yes	Yes	No	Don't know	Don't know	Don't know
6	Yes	51	F	74	34	50–99 times	No	No	No	No	Don't know	Know	Don't know
7	Yes	67	M	14	6	> 100 times	No	Yes	No	No	Don't know	Know	Know
8	Yes	52	M	82	87	> 100 times	No	No	No	No	Don't know	Know	Don't know
9	Yes	53	F	16	8	Don't want to answer	No	Yes	Yes	No	Know	Don't know	Don't know
10	Yes	60	M	16	6	> 100 times	Yes	No	Yes	No	Know	Know	Know
11	Yes	60	M	27	19	50–99 times	No	Yes	No	No	Don't know	Know	Don't know
12	Yes	53	M	20	18	1–45 times	Yes	No	No	No	Don't know	Know	Don't know
13	Yes	45	M	21	13	> 100 times	No	Yes	Yes	No	Don't know	Don't know	Don't know
14	Yes	58	M	21	10	50–99 times	No	No	No	No	Know	Know	Know
15	Yes	61	M	13	8	> 100 times	No	Yes	No	No	Know	Know	Know
16	Yes	50	M	14	11	50–99 times	No	Yes	Yes	No	Don't know	Know	Don't know
17	Yes	60	M	18	16	> 100 times	No	No	Yes	No	Know	Know	Know
18	Yes	73	M	16	12	> 100 times	No	Yes	No	No	Know	Know	Know
19	Yes	61	M	29	21	> 100 times	No	No	No	No	Don't know	Know	Don't know
20	Yes	65	F	22	14	50–99 times	No	No	Yes	No	Don't know	Don't know	Know
21	Yes	71	M	38	20	> 100 times	No	Yes	No	No	Don't know	Don't know	Don't know
22	No	42	M	18	11	-	No	No	Yes	No	Don't know	Don't know	Don't know

HBV, hepatitis B virus; HCV, hepatitis C virus; HCV-Ab, hepatitis C virus antibody; C.O.I, cut off index; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; Ct, cycle threshold; HBs-Ag, hepatitis B surface antigen; HBs-Ab, hepatitis B surface antibody; HBc-Ab, hepatitis B core antibody; LFT, liver function test; AST, aspartate transferase; ALT, alanine transferase; U/L, unit per liter. [†]HCV screening for donated blood has been introduced since 1989 in Japan.

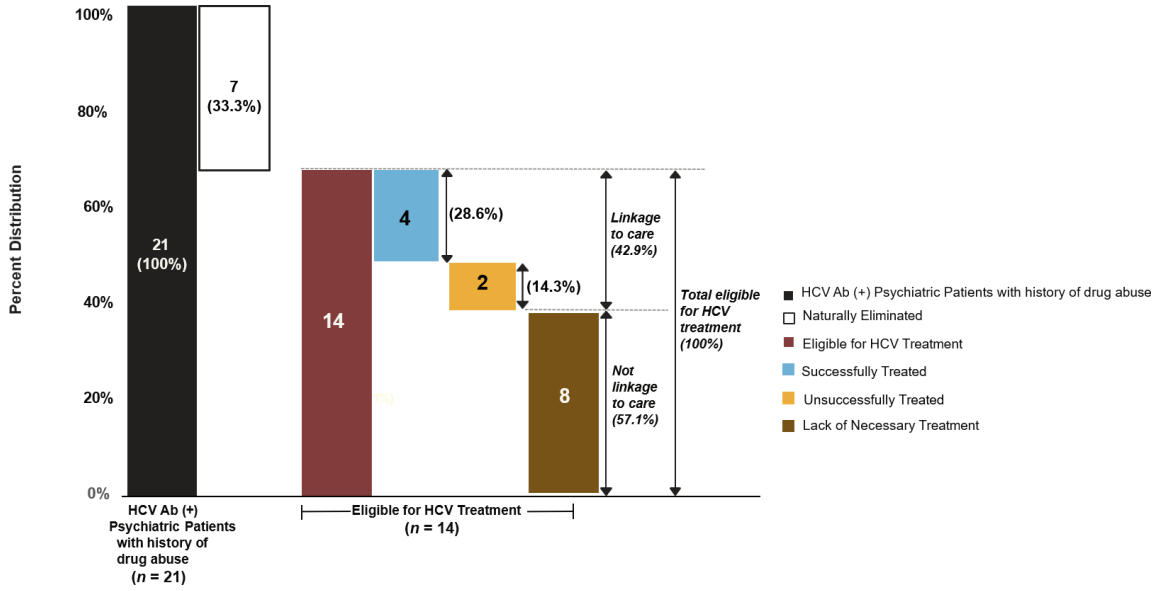

Figure 1. Percent distribution of linkage to care among HCV-Ab positive psychiatric patients with history of drug abuse. This figure represents among the 14 patients eligible for HCV treatment, 6 (42.9%) were linked to the care, and 8 (57.1%) were not linked to the care.

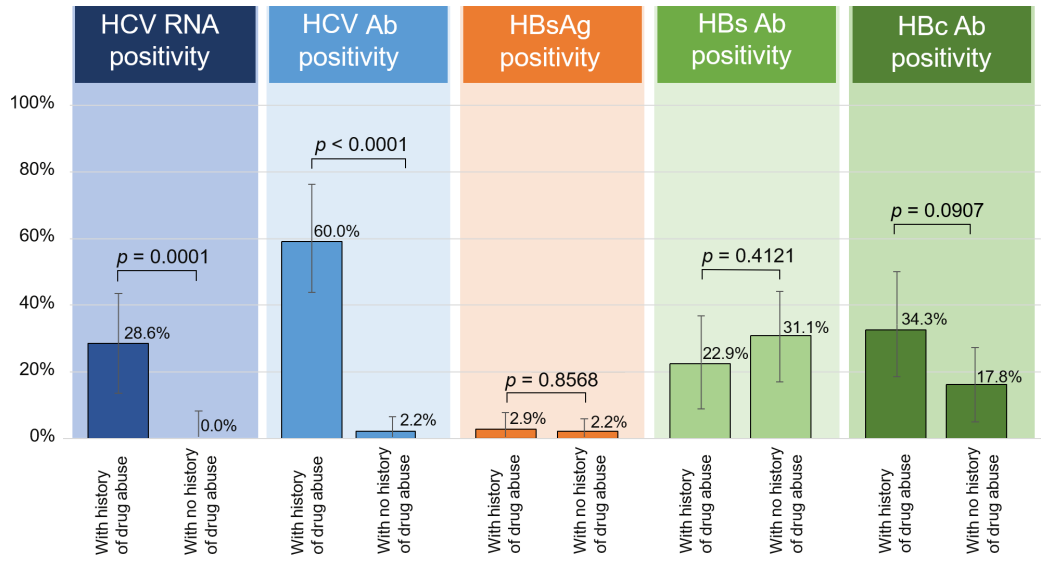

Figure 2. Hepatitis B and C virus infection status of psychiatric patients with history of drug abuse (n = 35) and no history of drug abuse (n = 45). This figure represents the prevalence of HCV-Ab and HCV RNA among psychiatric patients with a history of drug abuse was 60.0% (21/35, 95% CI: 43.8–76.2%) and 28.6% (10/35, 95% CI: 13.6–43.5%), respectively, which was significantly higher than in psychiatric patients without a history of drug abuse (HCV-Ab 2.2%, 95% CI: 0–6.5%, $p < 0.0001$; HCV RNA 0.0%, 95% CI: 0–8.2%, $p = 0.0001$). The prevalence of HBs-Ab was 22.9% (8/35, 8.9-36.8%), and that of HBc-Ab was 34.9% (12/35, 18.6–50.0%) among psychiatric patients with a history of drug abuse. There was no significant difference in the HBsAg positive rate between the two populations ($p = 0.8568$). However, the HBc-Ab positive rate tended to be higher in psychiatric patients with a history of drug abuse than in those without a history of drug abuse ($p = 0.0907$).

patients (60.0%) showed abnormal results for ALT and AST. (Table 2).

According to the questionnaire results, 8 out of 10 patients (80.0%) reported undergoing hepatitis virus screening tests during their hospital visits. The remaining 2 patients (20.0%) indicated that they had never undergone hepatitis virus testing before, but it was discovered that they had been tested without their knowledge due to their history of surgery or childbirth. Therefore, all HCV RNA-positive psychiatric patients with a history of drug abuse had undergone hepatitis virus testing at some point prior to their involvement in this study. Among them, 30.0% (3/10) received a detailed examination, while 20.0% (2/10) received HCV treatment.

HCV genotype

Out of the 10 patients who tested HCV RNA-positive, 5 were found to have genotype 1b (50%), 3 had genotype

2a (30%), and 2 had genotype 2b (20%) (Table 2). The HCV sequences of the 2 patients with genotype 2b (Case 3 and Case 4 in Table 2) showed a high degree of similarity at 98.8%.

Status of HBV infections

Only one psychiatric patient with a history of drug abuse (a 78-year-old male) was found to be positive for HBsAg (2.9%, 1/35, 95% CI: 0–8.4%). This patient was co-infected with HCV (Case 2 in Table 2), negative for hepatitis B surface antibody (HBs-Ab), and positive for hepatitis B core antibody (HBc-Ab), but HBV DNA was undetectable. The patient had undergone a hepatitis virus test during a previous medical visit and a subsequent detailed examination but did not meet the eligibility criteria for treatment. The prevalence of HBs-Ab was 22.9% (8/35, 8.9–36.8%), and that of HBc-Ab was 34.9% (12/35, 18.6–50.0%) among psychiatric patients with a history of drug abuse (Figure 2).

Among the non-drug abuser group, only one patient (female, 41 years old) was positive for HBsAg, with a prevalence of 2.2% (1/45, 95% CI: 0–6.5%). This patient tested negative for HBs-Ab and HBc-Ab and had undetectable levels of HBV DNA. She reported receiving a hepatitis virus test during a past visit to a medical institution but did not receive treatment due to the eligibility criteria not requiring further detailed examination. The positive rates of HBs-Ab and HBc-Ab were 31.1% (14/45, 95% CI: 17.6–44.6%) and 17.8% (8/45, 95% CI: 6.6–28.9%), respectively.

There was no significant difference in the HBsAg positive rate between the two populations (p = 0.8568). However, the HBc-Ab positive rate tended to be higher in psychiatric patients with a history of drug abuse than in those without a history of drug abuse (p = 0.0907, Figure 2).

Results from questionnaire

In this study, 62.9% (22/35) of psychiatric patients with a history of drug abuse and 37.8% (17/45) of psychiatric patients without a history of drug abuse reported prior HCV testing. Among psychiatric patients with no history of drug abuse (n = 27, after excluding 18 healthcare workers), the awareness of needle sharing as a route of transmission for HBV, HCV, and HIV was 51.9%, 48.1%, and 59.3%, respectively. In comparison, the corresponding figures for patients with a history of drug abuse were 42.9%, 65.7%, and 48.6%, respectively (p = 0.4816, p = 0.1646, p = 0.4030).

Discussion

We conducted a cross-sectional sero-epidemiological study at a psychiatric hospital in Japan to determine the prevalence of HBV and HCV infections among psychiatric patients with a history of drug abuse. This is crucial not only for their individual health but also for public health efforts in controlling the spread of viral hepatitis within their community. The prevalence of HCV-Ab and HCV RNA among patients with a history of drug abuse was found to be 60% and 28.6%, respectively, which were significantly higher than those in the control group without a history of drug abuse. The control group in this study had a high percentage of healthcare workers (40%). Despite healthcare professionals having a higher risk of HBV and HCV, their infection rate was lower, possibly due to increased infection prevention measures. In contrast, PWID may use drugs without prevention due to strong cravings.

Compared to studies conducted in Japan between 1992 and 1995, our study showed a decreased prevalence of HCV-Ab among drug users, with 60% in our study compared to 78.9% in 1992 and 74.5% in 1995 (*18-20*). According to an annual survey conducted from 1998 to 2017 in recovery support facilities for drug abusers, the HCV-Ab positivity rate was reported to be 30–50% (*20*). However, when compared to other countries, the prevalence of HCV-Ab in the Stockholm needle exchange program was reported to be 77%, and that of HCV RNA was 57% in 2018 (*21*). Our finding is consistent with a nationwide study conducted in Germany in 2020, where the prevalence of anti-HCV and HCV RNA among 2,466 opioid stimulation therapy (OST) patients was 58.8% and 27.3%, respectively (*22*).

According to studies conducted between 1995 and 2016, the prevalence of HCV-Ab among the general population in Japan was less than 1% (*6,23-25*). Therefore, our study revealed that the rate of HCV infection is extremely high among the population with a history of drug abuse in Japan. To achieve HCV elimination, a cascade of measures including awareness of hepatitis virus infection, risk reduction, access to screening and confirmatory testing, as well as linkage to treatment, are generally required to interrupt the chain of HCV transmission. As mentioned earlier, in Japan, measures such as hepatitis virus screening and subsidy programs for treatment have been implemented, effectively controlling infections in the general population (*3*).

However, has there been an opportunity for PWIDs to be tested and treated in Japan? There have been no reports on this matter. In our study, 21 out of 22 HCV-Ab positive patients were drug abusers, and all of them had been previously tested. Four of them were unaware that they had been tested, but it was determined based on their history of surgery, childbirth, or blood donation. In Japan, 60% of the general population have been tested for HCV, and 70% have been tested for HBV, including those who were unaware of it (*26*). Our study population of drug abusers receiving psychiatric treatment may not be representative of all drug abusers in Japan, as they have had access to medical care.

Our findings suggest that drug abusers who have had access to medical institutions in Japan have had good opportunities for hepatitis testing. However, access to treatment for those who test positive remains a concern. In our study, 33.3% of the 21 HCV-Ab positive drug abusers were considered to have naturally eliminated the virus, while the remaining 14 patients required treatment, but only 42.9% of them had been linked to medical care. In contrast, among HCV RNA positive individuals in the general population in Japan who survived from 2000 to 2015, 63.4–85.6% were linked to medical care by 2015 (*5*). In Japan, the introduction of direct-acting antiviral agents (DAAs) treatment in 2014 has led to a significant increase in the number of people receiving treatment as of 2022 (*27*). However, drug abusers still have a lower linkage to medical care compared to the general population. To meet the WHO target of hepatitis elimination by 2030, 80% of all eligible HCV patients must receive treatment (*2*). Worldwide, as of the end of 2019, it was estimated that only 21% of persons living with HCV knew their diagnosis, and among those diagnosed with chronic HCV infection, around 62% had been treated with DAAs (*2*). Linkage to treatment remains a challenge globally in achieving the WHO target, particularly among PWID. A study from Spain in 2018 found that only 45.9% of HCV patients with a history of drug use started treatment among 122 eligible patients (*28*), while an Italian focus group assessment reported that only 20.7% of HCV-positive patients among 3,796 eligible patients from 27 drug dependency centers were treated in 2019 (*29*).

For PWID, reinfection after treatment with DAAs has been a problem (*30-32*). To prevent new infections and reinfections, it is important to raise awareness about the risks of sharing needles. From this survey, 65.7% of psychiatric patients with a history of drug abuse recognized the risk of HCV infection due to sharing injection needles. Although it was slightly higher than the control group excluding healthcare workers, further improvement is desired. Many countries have implemented harm reduction programs recommended by WHO, such as syringe service programs (*33*). While such programs have not yet been introduced in Japan, there is a need for consideration in the future.

In this study, the prevalence of HBsAg positivity among individuals with a history of drug abuse was found to be 2.9%, which is higher than the reported prevalence of HBsAg positivity in the general population (0. 37%) (*5,6*). Although one patient each from those with history of drug abuse and those without was found to be HBsAg positive, our study is restricted to conduct the comparison between drug abusers and their control group in relation to HBsAg positivity and linkage to treatment. Meanwhile, HBsAg positivity did not change according to the presence or absence of drug abuse history, but the HBc-Ab positive rate was slightly higher in the drug abusers group, consistent with the previous report (*34*). It was suggested that the sharing of injection needles may have caused acute hepatitis B infection. Notably, awareness of the risks of needle sharing for HBV is slightly lower than for HCV, indicating a need for increased educational efforts to promote knowledge of these risks.

This study has several limitations. First, the study population consisted of individuals receiving treatment for drug addiction at a psychiatric hospital, which may introduce a bias towards those with a higher motivation for their own health. However, because PWID in Japan are often incarcerated or hidden in society, it can be challenging to conduct sero-epidemiological surveys, making drug users in psychiatric hospitals a feasible survey population. Additionally, the small sample size and single-center design are limitations of this study. Nevertheless, the absolute number of drug users in Japan is small and the psychiatric hospital included in this survey is one of the leading addiction treatment institutions in Japan. In situations where the target population is limited, small sample size studies can still provide valuable insights and serve as a starting point for further research in this underexplored area. With regards to the question of whether people with a history of drug abuse have access to hepatitis testing and treatment, the findings from this study cannot be completely generalized. Nonetheless, it was found that these individuals had been tested for hepatitis during their medical visits. However, these populations tend to have a lower linkage to treatment than the general population, which remains a challenge. Improving post-screening referral to treatment is crucial for them, going beyond the strategies employed for the general population. Additional efforts are needed to enhance the continuum of care for this rare and limited group, ensuring that they receive appropriate treatment and support. Despite the limitations described above, this study is a rare and valuable report that provides insights into the infection status of a population with a history of drug abuse in Japan.

Conclusion

Despite the small population of drug abusers in Japan, the high prevalence of HCV among them underscores the importance of addressing viral hepatitis in this group. Our study found that medical institutions in Japan provide opportunities for HBV/HCV testing for psychiatric patients with a history of drug abuse, but linkage to treatment is lower compared to the general population. Therefore, it is crucial to improve post-screening referral to treatment for drug abusers, beyond that of the general population. Strengthening collaboration between psychiatric hospitals and hepatologists is desirable. Overlooking this issue is not an option in the pursuit of hepatitis elimination.

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ORIGINAL ARTICLE

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A clinical decision rule to exclude central vertigo in the emergency department: A prospective, multicenter, observational study

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Abstract: To ensure good outcomes in patients presenting with vertigo, accurate prediction ruling out central vertigo is crucial during initial assessment. This study was conducted to develop a clinical decision rule (CDR) using objectively measurable predictors to exclude central vertigo, while maintaining 100% sensitivity. This was a multicenter, prospective, cohort study analyzing patients presenting to the emergency departments of six hospitals in Japan from April 2011 to March 2014. Eligible patients were 3,001 patients aged > 15 years. Patients were excluded if they presented with trauma, intoxication, heatstroke, anaphylaxis, or unconsciousness. The main outcome measure, definitive diagnosis of central vertigo, was based on confirmation of intracranial bleeding on head computed tomography (CT) or cerebral or cerebellar infarction or tumor on brain magnetic resonance imaging (MRI). Univariate analysis and multivariate recursive partitioning analysis were performed. A total of 1,938 patients were enrolled. Of 1,133 cases, 60 were diagnosed with central vertigo. The CDR diagnosed central vertigo if any of the following were present: headache or neck pain, vomiting, sBP > 150 mmHg, BS > 140 mg/dL, or LDH > 230 IU/L, providing sensitivity of 100% (95% CI 94.0–100%) and specificity of 21.2% (95% CI: 18.9–23.7%) to exclude central vertigo. The rule was validated in 805 eligible patients, of whom 87 had central vertigo, demonstrating sensitivity of 100% (95% CI: 95.8–100%) and specificity of 20.0% (95% CI: 17.4–22.9%). A highly sensitive CDR to exclude central vertigo was developed for patients presenting with vertigo to emergency departments. Further verification is needed to generalize this CDR.

Keywords: emergency department management, clinical research, common disease, decision making, dizziness

Introduction

Vertigo is a relatively frequent symptom, occurring in about 5% of emergency patients. Of these, about 3% are thought to have central vertigo, which is almost always due to cerebrovascular accidents involving the cerebellum or brainstem (*1,2*). Overlooking central vertigo may result in death, serious complications, or sequelae (*3*). Accurate detection is crucial in managing patients who present with vertigo to the emergency department (ED). Therefore, many studies have been conducted with the aim of not overlooking or picking up central vertigo. Almost all clinical decision rules can be used only when the presence or absence of neurological abnormal findings can be discriminated such as HINTS (head-impulse, nystagmus, test of skew) for acute vestibular syndrome which is becoming mainstream (*4-6*). Also in "Standard neurotherapy: Vertigo" published by the Japanese Society of Neurotherapy in 2020, the diagnosis of central vertigo or peripheral vertigo was based on the judgment of neurological findings, and there was a description that used many photographs and figures (*7*). Whereas the presence or absence of neurological findings is important, observations are greatly affected by the level of clinical skill of the attending medical staff. In fact, there is a report of meta-analysis that showed HINTS varied in accuracy when used by trained neurologists and by emergency physicians alone, and was not accurate enough to rule out stroke when used by emergency physicians alone (*4*).

Therefore, the development of clinical decision rules (CDRs) that provide high sensitivity for central vertigo

derived only from objective findings and tests without neurological findings that can be used by inexperienced physicians is very useful in the ED. However, there are few reports of predictors of central dizziness without neurological findings.

The present study was conducted as part of the Emergency Medicine, Registry Analysis, Learning and Diagnosis (EMERALD) project, aimed at minimizing life-threatening diseases being overlooked in EDs in Japan. The objective of this study was to develop a CDR, known as the EMERALD Vertigo Rule, using objectively measurable predictors to exclude central vertigo, while maintaining 100% sensitivity and offering as high a specificity as possible.

Materials and Methods

Study design

This multicenter, prospective, cohort study was conducted in the EDs of six general hospitals in Japan from April 2011 to March 2014. A total of 3,001 patients, aged > 15 years, presenting with a chief complaint of vertigo were considered for enrolment. Patients who presented with vertigo due to trauma, intoxication from drugs or alcohol, heatstroke, anaphylaxis, and those who were unconscious at the beginning of assessment were excluded.

All patient assessments were performed by residents supervised by staff physicians or attending emergency physicians. Physicians were oriented to the study and instructed to enter clinical findings at the time of assessment into data collection software specially developed by the EMERALD project on a smartphone device.

To minimize interobserver differences and observer biases, the focus was on objectively measurable data such as age, sex, heart rate, systolic blood pressure (sBP) and diastolic blood pressure (dBP), and temperature, which were defined as the first reading by the attending nursing staff. Data on symptoms that were clearly distinguishable as being present or absent and past medical history from the patient interview were gathered.

A variety of data from blood samples, such as blood sugar (BS), serum transaminase, serum lactate dehydrogenase (LDH), serum sodium, serum potassium, hemoglobin concentration, white blood cell (WBC) counts, and platelet counts were also collected, since these factors needed only a small amount of blood to measure. Only routine examination modalities applied to emergency patients in Japanese EDs were used, and all results were obtainable within 30 min.

All patient data were anonymized before being uploaded to the internet server *via* direct smartphone connection. Collected anonymized data were monitored and cleaned by the Joint Center for Researchers, Associates and Clinicians (JCRAC), an authorized center for quality management of data. The final dataset for analyses was provided by JCRAC.

The primary outcome, central vertigo, was defined as vertigo caused by cerebrovascular disease or tumor as detected by cranial computed tomography (CT) and/or brain magnetic resonance imaging (MRI)/magnetic resonance angiography (MRA). These were interpreted by emergency physicians, specialist neurological staff (neurologist or neurosurgeon), and/or radiologists. All participating hospitals were equipped with 64-row multidetector row CT scanners and an MRI device either in or close to the ED. CT was available within 1 h at all times. If the results appeared negative on plain CT and the patient was still suffering from vertigo, emergency physicians or residents hospitalized the patients and consulted neurological staff regarding whether the patients could be discharged.

Brain MRI/MRA might not be immediately available for these patients, depending on the situation. If the neurological staff suggested the patient would not need hospitalization, but the patient was still suffering from vertigo, the patient was admitted for observation and possible further intervention, as appropriate. Discharged patients were evaluated by outpatient follow-up or telephone interview.

Data from the Center Hospital of the National Center for Global Health and Medicine (NCGM) were defined as the derivation dataset, and data from the other five hospitals as the validation dataset. Two groups were compared: the central vertigo group (CV group) and the non-central vertigo group (non-CV group), in whom vertigo was not due to central vertigo. Univariate analyses were used to determine the strength of the association between each possible predictor variable and the outcome variable.

To develop a CDR, previously established methodological standards were followed (*8*). First, categorical variables showing values of $p < 0.05$ on univariate analyses were selected. Then, continuous variables showing values of $p < 0.05$ on univariate analyses were selected as clinically important possible predictors. Cut-offs for the selected, objectively measurable predictors were determined by receiver-operating characteristic (ROC) curve analyses.

Continuous variables were converted into categorical variables by the cut-offs. Setting the presence (1) or absence (0) of central vertigo as the outcome variable, multivariate, recursive partitioning analysis was performed to develop rules using only the selected and converted categorical variables. Sensitivity and specificity were estimated for each rule. Because a CDR for a life-threatening event such as central vertigo requires sensitivity of 100% with a narrow confidence interval, the practical rule with the highest specificity was selected. The CDR was verified using the validation dataset to determine the internal stability of the rule, and the sensitivity and specificity were calculated.

The research ethics board at each participating hospital approved the study protocol, which was designed in accordance with the STROBE C statement for observational studies. All procedures followed in this study were in accordance with institutional guidelines. Informed consent was obtained from all patients.

Statistical analysis

Wilcoxon's rank-sum test was used for continuous variables, and Fisher's exact test was used for categorical variables and multivariate recursive partitioning analysis to develop the CDR. Statistical analyses were performed using JMP V.11.2.1 software (SAS Institute, Cary, NC, USA).

Clinical Trial Registration

UMIN-CTR Clinical Trial-URL: *http://www.umin.ac.jp/ctr/index.htm*. Unique ID issued by UMIN: UMIN000004864

Results

A total of 1,236 consecutive patients were enrolled as the derivation dataset, and 1,765 consecutive patients were enrolled as the validation dataset. In the derivation dataset, exclusion criteria applied to 49 patients, whereas primary outcomes for 54 patients could not be confirmed without follow-up evaluation or telephone interview. The study flow for the 1,133 eligible patients is shown in Figure 1A. In the validation dataset, 1,764 patients were enrolled, of whom 14 patients were excluded according to the exclusion criteria. In a further 930 patients, primary outcomes could not be confirmed without a follow-up evaluation or telephone interview, whereas 16 patients had missing principal data; thus, 805 patients were eligible (Figure 1B). There were 60 central vertigo patients in the derivation dataset and 87 in the validation dataset.

Table 1 shows the classification by the causative disease and the percentage of patients whose medical images were inspected in the CV group (n = 60) and the non-CV group (n = 1,073) from among the enrolled patients in the derivation dataset. Cerebellar infarction (n = 30; 50.0%), cerebellar hemorrhage (n = 7; 11.7%), and brainstem infarction (n = 7; 11.7%) were the top three diseases in the CV group, whereas most cases of peripheral vertigo (n = 558; 52.0%) occurred in the non-CV group. CT was performed in 100% of the CV group and 72.8% of the non-CV group. MRI was performed in 53.3% of the CV group and 11.2% of the non-CV group.

Three patients (5.0%) in the CV group and 890 patients (82.9%) in the non-CV group were discharged from the ED. One of the three patients was initially diagnosed with peripheral dizziness as an emergency

Table 1. Classification by causative pathology and percentage undergoing imaging investigation in the CV group (n = 60) and non-CV group (n = 1,073) in the derivation dataset

Causative pathology	CV group (n = 60)
Cerebellar infarction	30 (50.0%)
Cerebellar hemorrhage	7 (11.7%)
Brainstem infarction	7 (11.7%)
Brainstem hemorrhage	2 (3.3%)
Other cerebrovascular diseases	12 (20.0%)
Brain tumor	2 (3.3%)
Imaging investigation	
Head CT	60 (100.0%)
Brain MRI/MRA	32 (53.3%)
Causative pathology	non-CV group (n = 1,073)
Peripheral vertigo	558 (52.0%)
Psychiatric disorders	112 (10.4%)
Neuroregulatory disorders	58 (5.4%)
Dehydration / Infectious disease	39 (3.6%)
Anemia / Gastrointestinal bleeding	20 (1.9%)
Hypertensive emergency	16 (1.5%)
Cardiovascular disease	14 (1.3%)
Electrolyte abnormality	11 (1.0%)
Others	245 (22.8%)
Imaging investigation	
Head CT	781 (72.8%)
Brain MRI/MRA	120 (11.2%)

CV, central vertigo; CT, computed tomography; MRI/MRA, magnetic resonance imaging/magnetic resonance angiography.

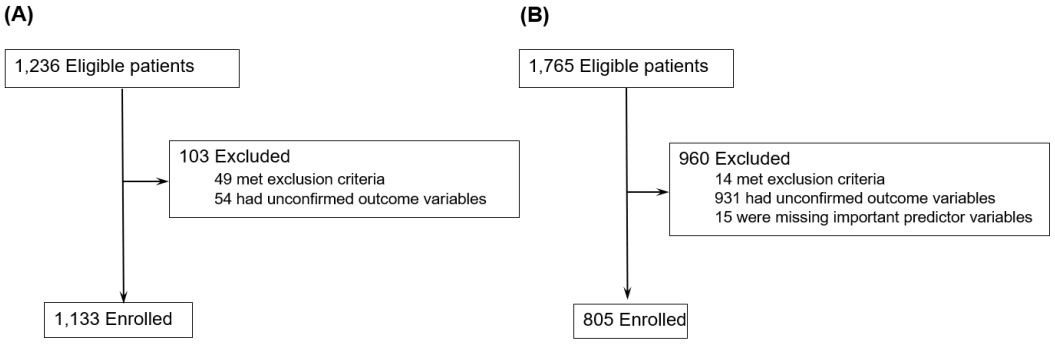

Figure 1. Flowchart of (A) the derivation dataset and (B) the validation dataset.

outpatient, but was finally diagnosed with central vertigo after consultation with an otolaryngologist. It was discovered via telephone interview that two patients, who did not undergo MRI or MRA, were initially diagnosed with peripheral vertigo and were discharged from EDs, but were then hospitalized elsewhere due to cerebellar infarction.

Table 2 shows the results of univariate analyses of the derivation dataset. The proportions of male patients and of patients with headache or neck pain and vomiting were significantly higher in the CV group than in the non-CV group. The proportion of patients with hypertension was significantly higher in the CV group, but there were no significant differences between the CV group and the non-CV group in the past medical or surgical history. Regarding vital signs and blood analyses, sBP, dBP, BS level, LDH level, and WBC count were higher in the CV group.

The cut-off values for continuous variables (sBP, dBP, BS level, LDH level, and WBC count) were determined using ROC curves. They were significantly higher in the CV group. Continuous variables were converted to categorical variables based on the cut-off value. These five converted variables and three categorical variables (including sex, vomiting, and headache and/or neck pain) were used for recursive partitioning.

As a result of the recursive partitioning analysis of the 1,133 enrolled patients, a CDR was developed using almost the same method as the EMERALD SAH rule (*9,10*), which is a CDR used to exclude the presence of subarachnoid hemorrhage in acute headache (Figure 2A).

Table 3 shows the comparison between the validation dataset of 718 eligible non-CV patients and the 945 excluded patients. In eligible patients, the median age was 69 years, and 36.8% were male, whereas in excluded patients, the median age was 68 years, and 36.5% were male. In addition, there were no significant differences in symptoms and measurable variables except for dBP and platelet counts between eligible non-CV patients and excluded patients.

The new CDR was verified with the validation dataset. Central vertigo was detected with sensitivity of 100% (95% CI: 95.8–100%) if the patient met any one of the following: presence of vomiting, headache or neck pain, sBP > 150 mmHg, BS > 140 mg/dL, or LDH > 230 IU/L. This CDR had a specificity of 20.0% (95% CI: 17.4– 22.9%) (Figure 2B).

As a result of the verification, the EMERALD

Table 2. Univariate correlations of variables with central vertigo

Characteristics	CV group (n = 60)	non-CV group (n = 1,073)	p value
Age (y)	65 (52–78)	63 (42–76)	0.07
Male sex	35 (58.3%)	446 (41.6%)	0.02
Symptom			
Headache or neck pain	19 (32.2%)*	197 (18.8%)*	0.02
Transient unconsciousness	1 (1.7%)*	59 (5.6%)*	0.37
Convulsions	0 (0.0%)*	7 (0.7%)*	1.00
Vomiting	36 (61.0%)*	392 (37.4%)*	< 0.01
Incontinence	1 (1.7%)*	7 (0.7%)*	0.36
Cochlear symptoms	4 (6.8%)*	146 (13.9%)*	0.17
Medical history			
Cerebral infarction	6 (10.0%)	69 (6.5%)*	0.29
Hypertension	32 (53.3%)	340 (32.2%)*	< 0.01
Dyslipidemia	7 (11.7%)	152 (14.7%)*	0.71
Diabetes mellitus	8 (13.3%)	105 (10.0%)*	0.38
Arrhythmia	3 (5.0%)	31 (3.0%)*	0.43
Neurological examination			
Cerebellar ataxia	19 (32.8%)*	18 (1.7%)*	< 0.01
Abnormal neurological findings	53 (89.8%)*	923 (92.0%)*	0.47
Vital signs			
Heart rate (bpm)	75 (67–87)	74 (66–84)	0.47
Systolic blood pressure (mmHg)	151 (137–180)	138 (119–160)	< 0.01
Diastolic blood pressure (mmHg)	87 (76–100)	76 (66–88)	< 0.01
Temperature (°C)	36.2 (35.8–36.7)	36.3 (36.0–36.7)	
Blood test results			
Blood sugar (mg/dL)	146 (118–175)	122 (104–146)	< 0.01
Serum lactate dehydrogenase (IU/L)	237 (203–267)	203 (178–239)	< 0.01
C-reactive protein (mg/dL)	0.08 (0.03–0.19)	0.06 (0.02–0.17)	0.10
Serum sodium (mEq/L)	139 (138–141)	140 (138–141)	0.28
Serum potassium (mEq/L)	3.8 (3.5–4)	3.8 (3.5–4)	0.98
White blood cell count (/μL)	7,900 (6,600–10,600)	6,900 (5,500–8,500)	< 0.01
Hemoglobin (g/dL)	14.1 (12.9–5.5)	13.6 (12.7–14.8)	0.07
Platelet count ($\times 10^4$/μL)	22.6 (18.9–26.9)	21.3 (17.7–25.4)	0.29

Continuous variables are indicated by medians (quartiles), nominal variables are indicated by numbers (proportion). CV, central vertigo. *: Percentages were calculated excluding those with missing data.

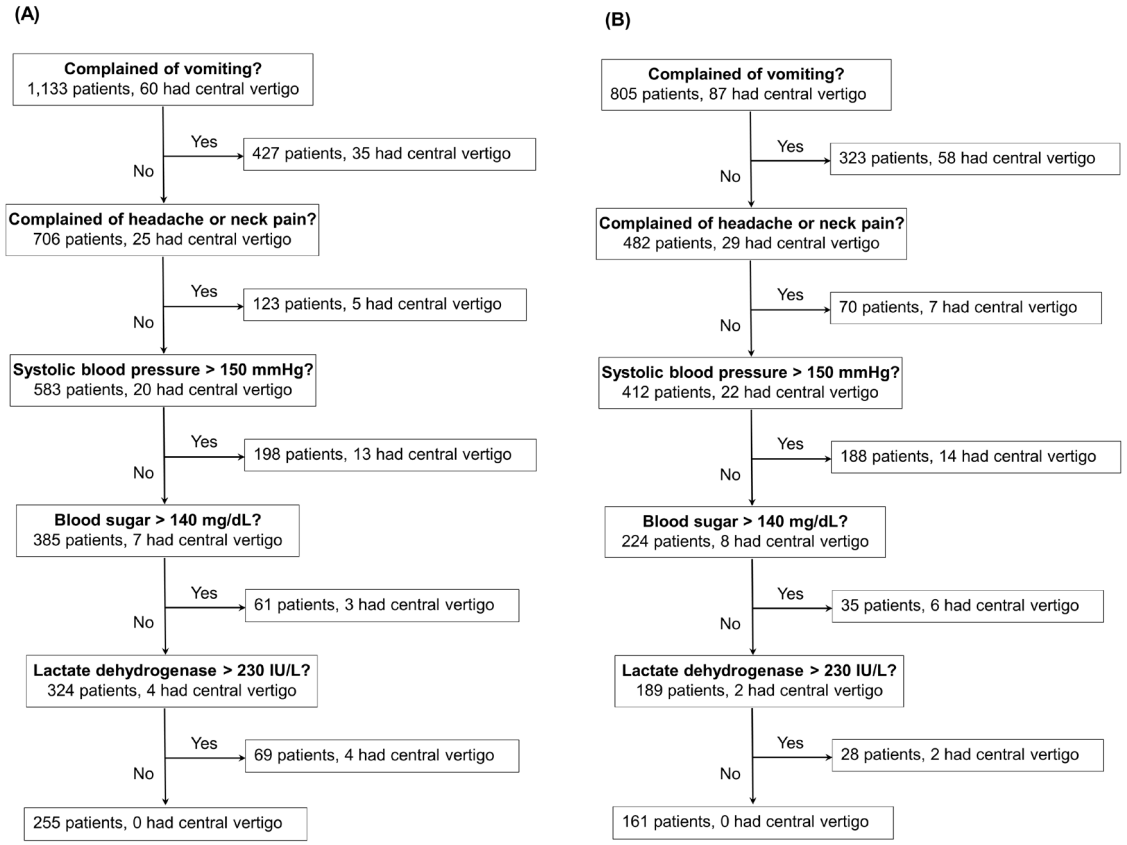

Figure 2. (A) A clinical decision rule excluding central vertigo with 100% sensitivity created using recursive partitioning, (B) Results of validation data using the created clinical decision rule.

Table 3. Comparison between validation data of 718 eligible non-CV patients and 945 excluded patients

Characteristics	Eligible non-CV patients (n = 718)	Excluded patients (n = 945)	p value
Age (y)	69 (58–77)	68 (57–76)	0.18
Male sex	264 (36.8%)	345 (36.5%)	0.75
Symptom			
Headache or neck pain	111 (15.5%)	168 (17.8%)	0.26
Transient unconsciousness	8 (1.1%)	21 (2.2%)	0.13
Convulsion	2 (0.3%)	7 (0.7%)	0.32
Vomiting	264 (36.8%)	396 (41.9%)	0.08
Incontinence	3 (0.4%)	3 (0.3%)	0.70
Cochlear symptoms	130 (18.1%)	147 (15.6%)	0.08
Medical history			
Cerebral infarction	65 (9.1%)	85 (9.0%)	0.86
Hypertension	288 (40.1%)	383 (40.5%)	1.00
Dyslipidemia	127 (17.7%)	80 (8.5%)	< 0.01
Diabetes mellitus	100 (13.9%)	107 (11.3%)	0.08
Arrhythmia	54 (7.5%)	111 (11.7%)	< 0.01
Neurological examination			
Cerebellar ataxia	24 (3.3%)	21 (2.2%)	0.21
Abnormal neurological findings	548 (76.3%)	599 (63.4%)	1.00
Vital signs			
Heart rate (bpm)	74 (66–86)	74 (65–83)	0.11
Systolic blood pressure (mmHg)	147 (128–67)	146 (128–164)	0.21
Diastolic blood pressure (mmHg)	82 (71–95)	80 (69–91)	< 0.01
Temperature (°C)	36.4 (36.0–36.7)	36.3 (35.9–36.6)	0.19
Blood test results			
Blood sugar (mg/dL)	126 (108–155)	132 (110–155)	0.10
Serum lactate dehydrogenase (IU/L)	214 (188–250)	214 (187–253)	0.67
C-reactive protein (mg/dL)	0.1 (0.04–0.2)	0.07 (0.03–0.17)	0.12
Serum sodium (mEq/L)	139 (138–141)	139 (138–141)	0.50
Serum potassium (mEq/L)	3.9 (3.6–4.2)	3.8 (3.6–4.2)	0.31
White blood cell count (/μL)	6,900 (5,400–8,600)	6,800 (5,400–8,400)	0.43
Hemoglobin (g/dL)	13.5 (12.6–14.4)	13.4 (12.3–14.3)	0.10
Platelet count ($\times 10^4$/μL)	20.7 (17.0–24.0)	21.9 (18.3–25.9)	< 0.01

Continuous variables are indicated by medians (quartile), nominal variables are indicated by numbers (proportion). CV, central vertigo.

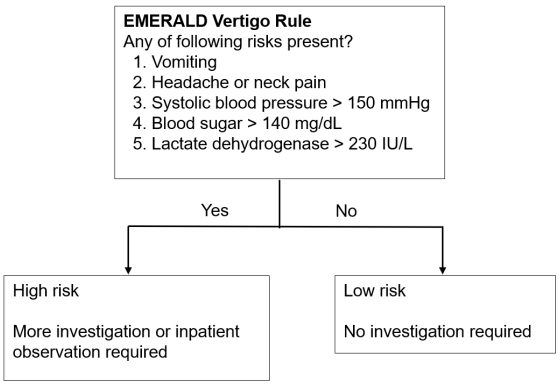

Figure 3. A proposed decision-making process for excluding central vertigo in vertigo patients.

Vertigo Rule was formulated as follows: in the presence of any of vomiting, headache or neck pain, sBP > 150 mmHg, BS > 140 mg/dL, or LDH > 230 IU/L, there is a possibility of central vertigo, and further examination or inpatient observation is warranted (Figure 3).

Discussion

Central vertigo may be overlooked in the initial management of vertigo patients in EDs. In EDs, many inexperienced residents are involved in patient management, and the reason for overlooking central vertigo was that the findings of these residents were likely less accurate and reproducible than those of neurological experts.

Kerber *et al.* reported that cerebrovascular disease was diagnosed in 3.2% (53/1,666) of patients presenting with vertigo. These patients were slightly older and more likely to be male than those without cerebrovascular disease (69.3 ± 11.7 *vs*. 65.3 ± 12.9 years, $p = 0.02$; 55% *vs*. 36% male, $p < 0.01$; respectively) (*2*) The present derivation dataset was compared with that of the report by Kerber *et al.* The percentage of central vertigo patients in the present study was significantly higher than the percentage in the report by Kerber *et al.* (5.6% *vs*. 3.2%, $p < 0.01$); however, the proportion of men in the non-CV group was consistent with their report. The age of patients tended to be higher in the CV group than in the non-CV group, but not significantly higher.

In the present study, the EMERALD Vertigo Rule was developed, with central vertigo suspected if any of the following five are present: vomiting, headache or neck pain, sBP > 150 mmHg, BS > 140 mg/dL, or LDH > 230 IU/L. It is not surprising that sBP and BS increase as stress increases, as in cerebrovascular disease, but the serum LDH value seems to be hardly related to stress. Prior to the 1990s, there were many reports that enzymes such as LDH in serum or cerebrospinal fluid increase significantly in stroke patients (*11-13*). These reports support LDH being one of the predictors used in the EMERALD Vertigo Rule.

The new CDR has 100% sensitivity to exclude central vertigo. Besides this, it was also ensured that the criteria were objectively measurable values that were easy to use during medical examinations in EDs. The sensitivity based on the validation dataset was also shown to be 100%; thus, the EMERALD Vertigo Rule seems to have a certain degree of external validity.

Despite being a prospective study, there were several deficits in the data. Simultaneous gathering of clean data proved difficult while emergency patient care was being provided. Some telephone numbers obtained were incorrect, and some patients did not have access to a telephone. Moreover, selection biases were likely given the large number of samples excluded. Missing validation data in 930 patients meant that primary outcomes could not be confirmed without follow-up evaluation or telephone interview, whereas 16 patients had missing principal data.

A total of 946 patients in whom primary outcomes could not be confirmed were those who were not diagnosed as having central vertigo during their initial medical examination in the EDs.

There were no significant differences in patients' characteristics, except dyslipidemia, arrhythmia, dBP, and platelet counts, between eligible non-CV and excluded patients (Table 3). Of the four significant characteristics (dyslipidemia, arrhythmia, dBP and platelet counts), the dBP, which was likely to be associated with the five parameters of the EMERALD Vertigo Rule, was significantly lower in the excluded patients than in the eligible non-CV patients. Since dBP was significantly higher in the CV group than in the non-CV group in the derivation data, a significantly lower dBP in excluded patients than in eligible non-CV patients did not increase the likelihood of including CV patients in excluded patients. It was not possible to directly prove that there were no patients with central vertigo among the excluded patients, but at least there were no relevant significant differences in the eligible non-CV patients and the excluded patients.

A meta-analysis by Robert Ohle *et al.* reported that HINTS when used by emergency physicians alone was not accurate enough to rule out stroke (*4*). Also, there are several reports that noted the need for training of emergency physicians to improve the accuracy of neurologic findings (*4,6*). In addition, we emphasized high sensitivity over low specificity, because ED care is focused on ruling out overt disease rather than making a definitive assessment. Although the EMERALD Vertigo Rule has less specificity, the focus was on creating more practical CDRs for inexperienced physicians who work extensively after-hours. This CDR seems to be very useful in practical clinical settings, because the rule is simple, and the predictors were shown to be not very subject to interobserver disagreement. Because the number of patients who undergo after-hours medical examinations continues to increase, it is also

quite useful that "only" about 20% of patients do not have to undergo detailed examinations and specialist consultations.

In conclusion, we propose that, when treating vertigo patients, further tests and follow-up inpatient observations are needed in patients who meet any of the following criteria: presence of vomiting, headache, or neck pain, sBP over 150 mmHg, BS over 140 g/dL, or LDH over 230 IU/L. The EMERALD Vertigo Rule may be useful in the initial management of emergency patients presenting with vertigo. Further validation will be required for its generalization.

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ORIGINAL ARTICLE

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Trends in prescription days and intervals between physician visits and their impact on glycemic control before and during the COVID-19 pandemic in Japan

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Abstract: We assessed trends in glycemic control among individuals with diabetes before and during the coronavirus disease 2019 pandemic. We used two databases to investigate changes in prescription days and the interval between physician visits and their impact on glycemic control among individuals with diabetes in Japan between 2017 and 2020. The analysis using the JMDC database indicated that prescription days were extended by approximately 20 days in 2020 compared to other years. The analysis using the Japan diabetes comprehensive database project based on an advanced electronic medical record system database revealed that intervals between physician visits were extended by approximately 7 days in 2020 compared to other years and glycemic control did not materially change in 2020. These results suggest that the prescription days increased with the spread of coronavirus disease 2019 in patients with diabetes, but the impact of the coronavirus disease 2019 pandemic on glycemic control appeared to be small.

***Keywords*:** diabetes, coronavirus, outpatient

Introduction

The spread of coronavirus disease 2019 (COVID-19) had a major impact on the treatment of lifestyle-related diseases. To reduce the risk of infection, efforts were made to discourage medical visits in many countries. In April 2020, the Japanese government declared a state of emergency and urged the public to avoid leaving home and visiting medical institutions unnecessarily. Because diabetes management requires continuous medical care (*1,2*) there have been concerns that extended intervals between physician visits may have negatively impacted glycemic control because individuals with diabetes had fewer opportunities for prescription days and lifestyle guidance.

According to a survey of 155 countries released by the World Health Organization in May 2020, 53% of the countries surveyed reported that some or all services for diabetes and diabetes-related complications were interrupted (*3*). Additionally, a study examining trends in diabetes-related services (HbA1c level, serum creatinine level, and urine protein level evaluations; fundus examinations; diabetic foot care; renal care services) in Japan reported a downward trend in diabetes-related medical care during the COVID-19 pandemic (*4*). Furthermore, changes in lifestyles and their associations with the metabolic and glycemic statuses among patients with diabetes during the first COVID-19-related state of emergency in Japan were reported (*5*). Some studies indicated that glycemic control was improved during the COVID-19 pandemic (*6*), while others showed deterioration (*7,8*) or no change (*9*). However, to the best of our knowledge, no study has examined trends in prescription adjustment and physician visit intervals during COVID-19 in patients with diabetes in relation to glycemic control.

To address this gap, we aimed to determine whether the intervals between physician visits lengthened and whether glycemic control deteriorated among those with diabetes during the COVID-19 pandemic. We first evaluated the prescription days using the JMDC Claims Database — a large-scale claims database in Japan encompassing populations across various regions of Japan, covering about 10 million people, with the

prescription days serving as a surrogate measure of physician visit intervals. We further investigated the interval between physician visits and glycemic control using the J-DREAMS (Japan Diabetes compREhensive database project based on an Advanced electronic Medical record System), a large-scale database directly linked to electronic medical records.

Materials and Methods

We obtained anonymized individual data from the JMDC Claims Database, which is a medical database managed by JMDC Inc. Japan has a universal health insurance system. Citizens are covered by one of several insurance systems, including employee health insurance programs, the national health insurance program, and the elderly health care system. The JMDC database contains the monthly claims reported by multiple employee health insurance programs since January 2005. This research using the JMDC database was approved by the institutional review board of the Yokohama City University (Reference No. B200800024).

We counted the median number of days between diabetes medication adjustments for each month from 2017 to 2020. Diabetes medication prescriptions were defined based on the World Health Organization Anatomical Therapeutic Chemical classification codes. Those with A10 codes (starting with A10X) were excluded. Those prescribed during hospitalization were also excluded. During the subgroup analysis, we categorized hospitals according to the number of beds (0-19, 20-99, 100-199, 200-299, 300-499, more than 500, and unknown).

To further examine the trends in intervals between prescription days and glycemic control for patients with diabetes who visited relatively large hospitals, we calculated the average intervals between physician visits and average HbA1c using the Japan diabetes comprehensive database project based on an advanced electronic medical record system (J-DREAMS) as of November 2021. The J-DREAMS included 65 participating facilities specialized in diabetes care. The research using the the J-DREAMS was reviewed and approved by the institutional review board of the National Center for Global Health and Medicine (approval number: NCGM-G-003329-00). The median intervals between physician visits were calculated for patients whose HbA1c was measured from 2017 to 2020 and for whom visit intervals could be estimated and compared between years. The interval between physician visits was estimated when HbA1c was measured at least once during the relevant year, and at least one HbA1c measurement was confirmed within approximately 180 days.

All statistical analyses were performed using R software (version 4.12 for Windows; R Project for Statistical Computing, Vienna, Austria).

Results

The JMDC database contained an average of 6,155,934, 6,963,009, 7,511,122, and 7,801,111 individuals each month during 2017, 2018, 2019, and 2020, respectively (Figure 1).

Results of all medical facilities showed that the median number of days between prescription days increased in February, March, April, and May of 2020 compared to those during other years (Figure 2). The analysis stratified by the number of beds showed no change in the median number of days between prescription days for medical institutions with 19 beds or less, but an increase for those with 20 or more beds, especially during April and May (Supplemental Figure S1, *https://www.ghmopen.com/site/supplementaldata.html?ID=80*).

The analysis involving the J-DREAMS included 7030 patients observed from 2017 to 2020. The mean intervals between physician visits were 53.8 days in

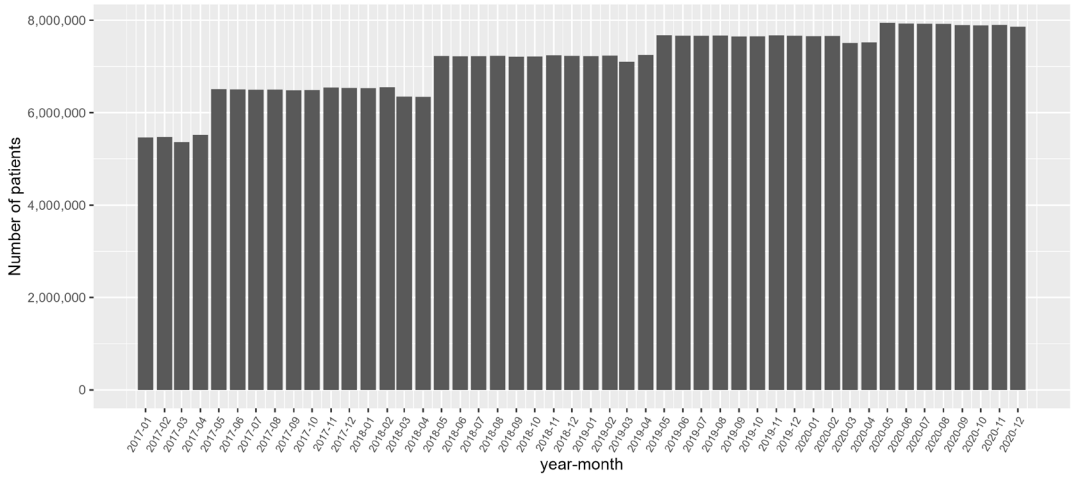

Figure 1. Number of patients during each month.

2017, 55.0 days in 2018, 55.7 days in 2019, and 62.1 days in 2020; however, the mean HbA1c values were 7.37 in 2017, 7.35 in 2018, 7.26 in 2019, and 7.30 in 2020 (Figures 3 and 4).

Discussion

During the JMDC database analysis, we investigated trends in prescription days among patients with diabetes in Japan. We found that these intervals were extended among patients who visited medical institutions with more than 20 beds during the COVID-19 pandemic. Another analysis involving the J-DREAMS database showed that although the intervals between physician visits were extended, the change in glycemic control was minimal before and during the COVID-19 pandemic.

These results suggest that the prescription days increased with the spread of COVID-19 among patients with diabetes, but that the impact on glycemic control was small among patients attending specialized diabetes care facilities. According to the analysis using the JMDC database, the prescription days for patients who visited large hospitals increased, suggesting that the role of medical resources may have been appropriately adjusted. Previous studies have shown that the number of physician visits decreased during the expansion of the COVID-19 pandemic among patients with diabetes (*10,11*). These results suggest that appropriate treatment continuation might have been achieved by reducing the number of visits and increasing the prescription days.

According to the results of the J-DREAMS study of patients who visited physicians at relatively large medical institutions, the interval between physician visits was extended an average of approximately 7 days, but the mean HbA1c levels remained unchanged. Therefore, the extension of approximately 7 days might have had little effect on glycemic control. Although previous studies have shown that the interval between physician visits during the COVID-19 pandemic was significantly

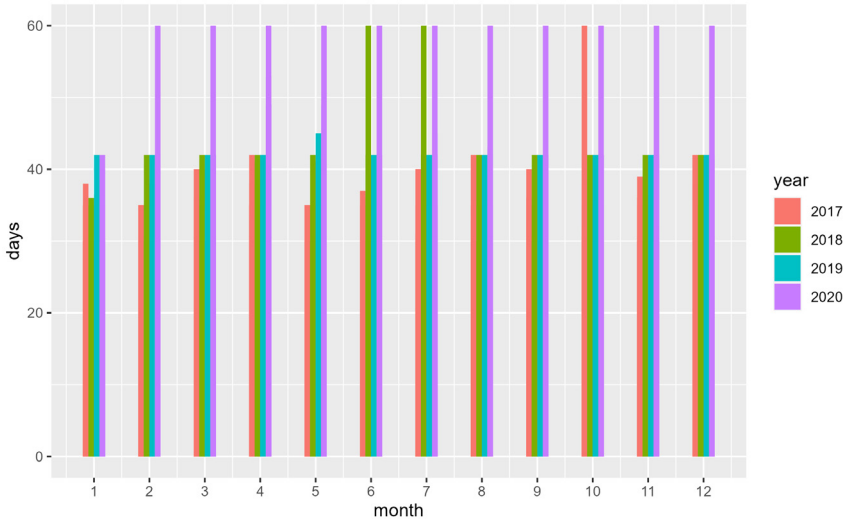

Figure 2. Changes in the median prescription days prescribed (in days) from 2017 to 2020 (stratified by the number of hospital beds).

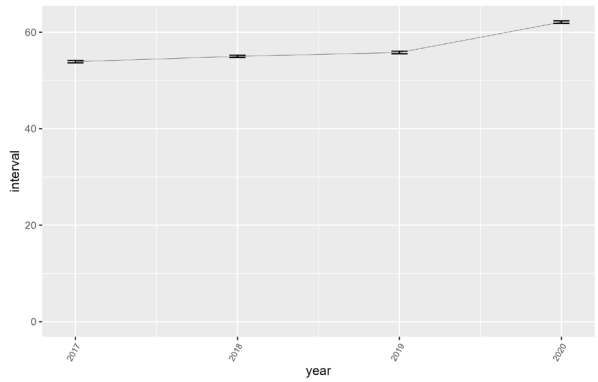

Figure 3. Mean interval between physician visits during each year (7,030 patients had data for all years).

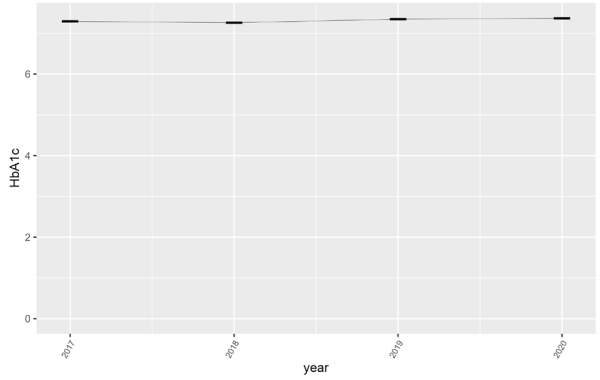

Figure 4. Mean HbA1c values (7,030 patients had data for all years).

associated with HbA1c control, worsening of HbA1c was not observed during the analysis involving the J-DREAMS (*12*). This may be because the association reported in the previous literature was observed for HbA1c < 7%, and the effect on HbA1c was minor during a short period of 7 days.

The J-DREAMS includes relatively large hospitals; however, the characteristics of large hospitals are similar to those of hospitals not included in the J-DREAMS. Therefore, by combining the results of the analysis using the J-DREAMS and those of the analysis using the JMDC database, it was observed that little effect on glycemic control occurred despite the increased prescription days and reduced number of physician visits.

This study had several limitations. First, because the JMDC database includes those enrolled in employment insurance, the survey was limited to individuals of working age and their dependents; it did not include populations not covered by corporate health insurance. Second, caution should be exercised when interpreting the data. Additionally, all medical institutions participating in the J-DREAMS are educational facilities designated by the Japan Diabetes Society, and the data included in this study are only those of patients who attended relatively large medical institutions. Therefore, we conducted an analysis using data from before 2020 to examine prescription durations and appointment intervals at that time. However, on May 8, 2023, COVID-19 has been downgraded to a Class V Infectious Disease under the Infectious Disease Act, resulting in the ability of a wide range of medical institutions to provide treatment for the infection (*13*). This change has brought about various transformations, including the normalization of routine medical responses. This shift is believed to have influenced public awareness of COVID-19. Further investigation is necessary to understand the implications of this reclassification on prescription durations and blood glucose control.

In conclusion, our results indicate that the interval between physician visits among patients with diabetes in Japan was extended during the COVID-19 pandemic, but the effect on HbA1c was small. Additionally, prescription days were extended for patients who visited medical facilities with fewer than 20 beds, suggesting that medical resources may have been appropriately distributed during the pandemic. These results have certain implications regarding diabetes management. If the patient's glycemic control status is stable, extending the prescription period as part of the treatment plan during the pandemic may be acceptable. Additionally, given the possibility of future pandemics, it may be worth exploring a system that allows for ongoing access to essential medications while minimizing the need for frequent visits to healthcare facilities. Such a system would help to reduce exposure risks while ensuring that those with chronic conditions can continue to receive necessary treatments.

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Conflict of Interest: SY is employed by Integrity Healthcare Co., Ltd., a company that provides online medical systems, in Japan. However, this study was not related to the company. Other autors declare they have no conflicts of interest concerning this manuscript.

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Difference in clinical courses and causes of COVID-19-related deaths in hospitalized patients infected with omicron and delta variants: A retrospective study in Japan

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Abstract: Despite the lower rate of severe illness associated with the omicron variant than the delta variant, more deaths have occurred among patients with mild-to-moderate COVID-19 in Japan since the omicron variant surge during the sixth wave. This study aimed to elucidate the background, clinical course, and causes of death in patients with COVID-19. We conducted a retrospective observational study on patients with COVID-19 admitted to the National Center for Global Health and Medicine who subsequently died during the delta (July–September 2021) and omicron variant outbreaks (December 2021–August 2022). Among the 20 patients who died during the delta variant epidemic, the main causes of death were pneumonia (n = 16, 80%), preadmission complications (n = 3, 15%), and complications occurring during hospitalization (n = 1, 5%). However, during the omicron variant epidemic, 7/24 patients (29%) died of pneumonia, 11 (46%) died of complications before admission, and 6 (25%) died of complications during admission. During the omicron variant outbreak, two-thirds of the COVID-19 deaths during hospitalization were not primarily caused by pneumonia, unlike the delta variant outbreak, during which pneumonia had a greater impact on mortality. As patient demographics and clinical pictures change, the establishment of medical infrastructure for patients with life-threatening comorbidities and careful monitoring of acute COVID-related complications are essential.

***Keywords*:** pneumonia, respiratory failure, comorbidities, disease progression, medical infrastructure, COVID-related complications

Introduction

The delta variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a higher severity rate than the previous variants (*1*). During the fifth wave, numerous severe COVID-19-related deaths were reported in Japan. The omicron variant has a lower severity rate than the delta variant (*2*). However, during the sixth wave and beyond, the number of deaths due to mild-to-moderate COVID-19 increased in Japan.

COVID-19 worsens complications like cardiovascular diseases, even in mild cases (*3*). In Japan, patients aged ≥ 60 years accounted for 74% of COVID-19 fatalities during the fifth wave (delta variant outbreak period). This rate increased to 95% during the sixth wave (omicron variant outbreak period), indicating that a higher proportion of older adults are affected (*4*). Nevertheless, a significant lack of understanding remains regarding the direct causes, background, and progression of COVID-19-related deaths, especially during the omicron variant outbreak.

Therefore, we conducted this retrospective observational study on patients with COVID-19 admitted to the National Center for Global Health and Medicine (NCGM) who subsequently died during both the delta and omicron variant outbreaks to elucidate the clinical

course and causes of death.

Patients and Methods

Age, sex, preexisting comorbidities, activities of daily living (ADL) status before hospitalization, COVID-19 vaccination history, complications during hospitalization, cause of death categorized according to the International Statistical Classification of Disease and Related Health Problems (ICD-10), and clinical course were compared and described for patients admitted to the NCGM during the delta (July 2021–September 2021) and omicron variant outbreak (December 2021–August 2022) periods, who were diagnosed with SARS-CoV-2 infection based on positive microbiological testing and subsequently died during hospitalization.

Additionally, we classified the causes of death into three groups: *i*) respiratory failure due to pneumonia, *ii*) exacerbation of preexisting comorbidities before hospitalization, and *iii*) complications that occurred during hospitalization. Clinical course descriptions of 5 representative cases are provided.

This study was reviewed and approved by the Ethics Committee of the Center Hospital of the National Center for Global Health and Medicine (NCGM-S-004634-00) on the condition that a document that declares an opt-out policy by which any patient and/or relatives could refuse to be included in this study was uploaded on the web page of the Center Hospital of the NCGM. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Among the patients with COVID-19 admitted during the delta and omicron variant outbreaks, 20 and 24 died during hospitalization, respectively. The median age (interquartile range) was 63.0 years (55.3–78.0) and 74.5 years (68.3–85.3), with 9 (45%) and 18 (75%) being males, respectively. Regarding COVID-19 vaccination history during the delta variant outbreak, 1 patient (5%) had received 2 doses, 2 patients (10%) had received 1 dose, 13 patients (65%) had no vaccination history, and 4 patients (20%) had unknown vaccination histories (Table 1). During the omicron variant outbreak, 4 patients (17%) had received 3 doses, 6 patients (25%) had received two doses, 8 patients (33%) had no vaccination history, and 6 patients (25%) had unknown vaccination histories.

During the delta variant outbreak, cause *i*) respiratory failure due to pneumoniae accounted for the death of 16 patients (80%), cause *ii*) exacerbation of preexisting comorbidities before hospitalization for the death of 3 patients (15%), and cause *iii*) complications that occurred during hospitalization for the death of 1 patient (5%). However, during the omicron variant outbreak, cause *i*) respiratory failure due to pneumoniae accounted for the death of 7 patients (29%), *ii*) exacerbation of preexisting complications for 11 patients (46%), and *iii*) complications during hospitalization for 6 patients (25%). Out of the 16 patients who died due to respiratory failure due to pneumonia during the delta variant outbreak and 7 patients during the omicron variant outbreak, chest computed tomography (CT) scans performed at the end of hospitalization revealed lesions primarily centered around the bronchi and its surroundings in 1 (6%) and 4 patients (57%) in the delta variant outbreak group. Meanwhile, lesions primarily centered around the lung interstitium were observed in 15 (94%) and 3 (43%) patients, respectively.

In addition to this classification of causes of death, we summarized the age, sex, underlying comorbidities, pre-hospitalization ADL, COVID-19 vaccination history, complications that occurred during hospitalization, and statistical classification of diseases, disabilities, and causes of death (ICD-10) for each patient in Table 1 and Table 2. In total, 5 representative cases were selected from the 3 causes of death groups, and their clinical courses are described below.

Case 1: Respiratory failure due to pneumonia (COVID-19 pneumonia)

A male in his 80s with a medical history of myocardial infarction treated with percutaneous coronary intervention, type 2 diabetes, and hypertension had trouble moving while in his home bathtub and was urgently transported to the hospital. The patient did not receive the COVID-19 vaccine. Upon initial assessment, he exhibited poor oxygenation. Treatment was initiated with high-flow nasal cannula therapy, 2 mg/kg methylprednisolone (no remdesivir was administered due to hepatic and renal dysfunction), heparin, and antibiotics. On the third day in hospital, his respiratory condition worsened, necessitating noninvasive positive pressure ventilation (NPPV) and the initiation of tocilizumab therapy. Despite receiving pulse therapy with methylprednisolone at a dose of 250 mg starting on the 11th day, the patient's symptoms did not improve, leading to the worsening of respiratory failure on the 33rd day after death.

Case 2: Respiratory failure due to pneumonia (aspiration pneumonia)

A male in his 90s had a history of pulmonary emphysema, right leg block, and disuse syndrome. While attending the day-care facility, the patient contracted COVID-19 and was admitted on the fourth day of illness with a diagnosis of severe COVID-19. Treatment was initiated with remdesivir, dexamethasone, and heparin. Due to difficulty with oral intake, the patient experienced recurrent episodes of aspiration pneumonia after admission. Intravenous fluid therapy was maintained, but

Table 1. Primary causes of death in patients with COVID-19 admitted during the delta variant outbreak, characteristics, preexisting comorbidities, and complications during hospitalization (*n* = 20)

Cause of death	Age (years)	Sex	Number of vaccinations	ICD-10	Preexisting ADL	Preexisting comorbidities	Oxygen therapy	Complications during hospitalization
i) Pneumonia (*n* = 16)	20s	F	None	Respiratory infections	Independent	Hypertension, diabetes, obesity	ECMO	Progression of respiratory failure due to pneumonia
	30s	M	None	Respiratory infections	Independent	Obesity	Mechanical ventilation	Progression of respiratory failure due to pneumonia, emphysema, and pneumothorax
	50s	M	None	Respiratory infections	Independent	Myocardial infarction, diabetes, obesity	Mechanical ventilation	Progression of respiratory failure due to pneumonia
	50s	M	None	Respiratory infections	Independent	Rheumatoid arthritis, hypertension, diabetes, obesity	Mechanical ventilation	Progression of respiratory failure due to pneumonia
	50s	M	None	Respiratory infections	Independent	None	Mechanical ventilation	Progression of respiratory failure due to pneumonia
	50s	F	None	Respiratory infections	Independent	Diabetes, schizophrenia	Mechanical ventilation	Progression of respiratory failure due to pneumonia, *Pseudomonas aeruginosa* bacteremia
	60s	M	None	Respiratory infections	Independent	Nephritis in childhood	Mechanical ventilation	Respiratory failure progression due to pneumonia, pneumothorax, pyothorax, multiple organ failure
	60s	F	None	Respiratory infections	Independent	Diabetes, obesity	Mechanical ventilation	Respiratory failure progression due to pneumonia, mediastinum emphysema
	70s	M	None	Respiratory infections	Independent	Hypertension, atrial fibrillation, bronchial asthma	NPPV (No intubation request)	Respiratory failure progression due to pneumonia, atrial fibrillation, cerebral infarction, splenic infarction, gastrointestinal bleeding (under heparin use
	70s	M	2	Respiratory infections	Independent	Lymphoma, prostate cancer, hypertension	Mechanical ventilation	Respiratory failure progression due to pneumonia, suspected aspiration, pneumothorax
	70s	F	None	Respiratory infections	No record	None	NPPV (There was no key person, and due to the lack of expected improvement, intubation was not performed.)	Progression of respiratory failure due to pneumonia
	70s	F	None	Respiratory infections	Independent	Hypertension, hyperlipidemia, osteoporosis	HFNC (No intubation request)	Progression of respiratory failure due to pneumonia
	70s	F	None	Respiratory infections	Independent	Hypertension, HCV hepatitis	NPPV (No intubation request)	Progression of respiratory failure due to pneumonia
	70s	F	None	Respiratory infections	No record	None	NPPV	Respiratory failure progression due to pneumonia, malignant genital bleeding (under heparin use)
	70s	F	None	Respiratory infections	Daily support by helpers	SLE, Sjögren's syndrome, atrioventricular block	Mask	Aspiration pneumonia, pyelonephritis
	90s	M	1	Respiratory infections	Independent	After cholecystectomy	Reservoir mask (No intubation request)	Progression of respiratory failure due to pneumonia

Abbreviations: ADL, Activities of daily living. SLE, Systemic lupus erythematosus. ECMO, Extracorporeal membrane oxygenation. NPPV, Non-invasive positive pressure ventilation. HFNC, High-flow nasal cannula.

Table 1. Primary causes of death in patients with COVID-19 admitted during the delta variant outbreak, characteristics, preexisting comorbidities, and complications during hospitalization (*n* = 20) (continued)

Cause of death	Age (years)	Sex	Number of vaccinations	ICD-10	Preexisting ADL	Preexisting comorbidities	Oxygen therapy	Complications during hospitalization
ii) Preexisting comorbidities (*n* = 3)	30s	M	Unknown	Others	Independent	Acute traumatic subdural hematoma, cervical spine fracture (at the time of admission)	None (incubation only)	–
	50s	F	1	Respiratory failure	No record	Alcoholic cirrhosis (Child-Pugh C), esophageal varicose vein rupture, obesity	NPPV	Hypovolemic shock due to low albumin
	80s	F	Unknown	Cardiovascular diseases	Walk with a cane, eat without assistance	Rheumatoid arthritis, hypertension, acute myocardial infarction (at the time of admission)	None	–
iii) Complications during hospitalization (*n* = 1)	50s	F	None	Others	Independent	None	ECMO	Respiratory failure progression due to pneumonia, cerebral hemorrhage (under ECMO)

Abbreviations: ADL, Activities of daily living. SLE, Systemic lupus erythematosus. ECMO, Extracorporeal membrane oxygenation. NPPV, Non-invasive positive pressure ventilation. HFNC, High-flow nasal cannula.

the patient died on the 26th day due to respiratory failure caused by aspiration pneumonia.

Case 3: Exacerbation of preadmission complications

A woman in her 80s presented with severe valvular heart disease, chronic heart failure, and a history of stroke. The patient had received 3 doses of the COVID-19 vaccine. The patient was transported to our facility because of respiratory distress and was diagnosed with worsening chronic heart failure caused by severe valvular heart disease. Upon admission, the patient tested positive for SARS-CoV-2 by polymerase chain reaction. Respiratory failure, believed to be associated with heart failure, progressed, and the patient died on the fourth day of illness.

Case 4: Exacerbation of preadmission complications

A man in his 60s with stage 4 advanced small cell lung cancer received supportive care. He called for emergency assistance because of respiratory distress and was hospitalized. Although the patient had planned to be transferred to a hospice, on the 6th day of illness the patient came in close contact with a patient with COVID-19, and on the 7th day, the patient was diagnosed as an asymptomatic carrier of SARS-CoV-2. The patient was treated with a 3-day course of remdesivir to prevent severe disease progression. The patient experienced severe cancer-related pain, nausea, and fatigue, and sedation was initiated on the 8th day. The patient died on the 11th day.

Case 5: Complications during hospitalization

The patient was a male in his 80s with a history of hypertension, atrial fibrillation, and chronic obstructive pulmonary disease. The patient had received 2 doses of the COVID-19 vaccine. The patient developed a fever and cough, which led to hospital admission on the fourth day of illness with a diagnosis of moderate COVID-19 and chronic interstitial pneumonia. Remdesivir and antibiotics were administered. The following day, the patient required supplemental oxygen, and methylprednisolone treatment (250 mg) was initiated. However, pneumonia worsened, and pulse therapy with 1 g methylprednisolone and various immunosuppressive agents (cyclophosphamide, bortezomib, and tacrolimus) were administered sequentially. On the 11th day of illness, the patient developed heart failure, which improved with the initiation of diuretics and NPPV. On the 24th day, the patient was diagnosed with acute exacerbation of heart failure and hypotension with ST-elevation myocardial infarction. Due to respiratory distress, coronary artery catheterization was not performed, and the patient died on the 26th day due to worsening heart failure.

Table 2. Primary causes of death in patients with COVID-19 admitted during the omicron variant outbreak, characteristics, preexisting comorbidities, and complications during hospitalization (*n* = 24)

Cause of death	Age (years)	Sex	Number of vaccinations	ICD-10	Preexisting ADL	Preexisting comorbidities	Oxygen therapy	Complications during hospitalization
i) Pneumonia (*n* = 7)	60s	M	2	Respiratory infections	Independent	Hypertension, chronic kidney disease	NPPV	Pneumothorax, mediastinal emphysema
	70s	M	2	Respiratory infections	Independent	Diabetes, lung squamous cell carcinoma, MPA, chronic kidney disease	HFNC	Aspiration pneumonia, *Pseudomonas aeruginosa* pneumonia, acute myocardial infarction
	80s	F	Unknown	Respiratory infections	Requiring long-term care level 3*, walk with supervision	Rheumatoid arthritis, Parkinson's disease, after heart valve replacement	NPPV	Aspiration pneumonia, pulmonary pyogenic disease
	80s	M	None	Respiratory infections	Independent	Hypertension, diabetes, myocardial infarction, dyslipidemia	NPPV	Disseminated intravascular coagulation syndrome, multiple organ failure
	80s	M	Unknown	Respiratory infections	Needs long-term care level 4, requiring partial assistance with bathing and dressing	Normal pressure hydrocephalus, postoperative bladder cancer, angina pectoris, dementia	HFNC	Aspiration pneumonia
	90s	M	None	Respiratory infections	Cognitive impairment, requiring long-term care level 2	Emphysema, right bundle branch block	Reservoir mask	Aspiration pneumonia
	90s	M	2	Respiratory infections	Walking with assistance, requiring long-term care level 2	Hypertension, cerebral infarction, chronic kidney disease	Nasal cannula	Aspiration pneumonia
ii) Preexisting comorbidities (*n* = 11)	40s	M	Unknown	Others	Unknown	Alcoholic cirrhosis	None	Rupture of varicose vein in colon
	50s	M	3	Cardiovascular diseases	Independent	Sarcoidosis, chronic kidney disease	HFNC	Exacerbation of heart failure
	50s	M	None	Others	Independent	Polycystic Kidney, chronic kidney disease	None	MSSA bacteremia, uremia
	60s	M	Unknown	Malignant neoplasms	Requiring long-term care level 3	HIV infection, multiple metastases of gastric cancer, diabetes, hypertension	Nasal cannula	Hemorrhage from tumor, anemia
	60s	F	Unknown	Malignant neoplasms	Unknown	Multiple metastasis of rectal cancer, hypertension	None	Hemorrhage from tumor
	60s	M	None	Malignant neoplasms	Unknown	Multiple metastases of small cell lung cancer, hypertension	None	
	70s	M	Unknown	Malignant neoplasms	Unknown	Multiple metastases of liver cancer, heart failure	Nasal cannula	Exacerbation of heart failure

Table 2. Primary causes of death in patients with COVID-19 admitted during the omicron variant outbreak, characteristics, preexisting comorbidities, and complications during hospitalization (*n* = 24) (continued)

Cause of death	Age (years)	Sex	Number of vaccinations	ICD-10	Preexisting ADL	Preexisting comorbidities	Oxygen therapy	Complications during hospitalization
	70s	M	None	Malignant neoplasms	Independent	Transverse colon cancer multiple metastases, Bowel obstruction, Obesity	Nasal cannula	Transverse colon ileus, multiorgan failure
	70s	F	3	Others	Independent	Diabetes, extensive cerebral infarction	Nasal cannula	Brain herniation
	80s	F	3	Cardiovascular diseases	Independent	Valvular disease, heart failure, cerebral infarction	NPPV	Exacerbation of heart failure
iii) Complications during hospitalization (*n* = 6)	90s	F	3	Cardiovascular diseases	Requiring long-term care level 4, Walking only indoors with assistance	Valvular disease, heart failure, chronic kidney disease, hypertension	None	Acute pyelonephritis, exacerbation of heart failure, renal failure
	60s	M	2	Others	Independent	Obesity, hypertension	NPPV	Iliopsoas hematoma, hemorrhagic shock, acute renal failure
	60s	M	2	Cardiovascular diseases	Independent	Esophageal cancer, hypertension, transient ischemic attack	None	Aortic rupture
	70s	M	None	Others	Independent	Interstitial pneumonia, thymoma	Mechanical ventilation	Intracranial hemorrhage
	70s	M	None	Respiratory infections	Independent	Hypertension, diabetes	Mechanical ventilation	Bacteremia, septic shock, acute respiratory distress syndrome, acute renal dysfunction, ventilator-associated pneumonia
	80s	M	2	Cardiovascular diseases	Requiring long-term care level 2, requires partial assistance with excretion	Interstitial pneumonia, chronic obstructive pulmonary disease, atrial fibrillation, hypertension	NPPV	Acute myocardial infarction
	90s	F	None	Others	Requiring long-term care level 5, use a wheelchair	Polymyalgia rheumatoid arthritis, bullous pemphigoid, cerebral infarction	Reservoir mask	Bacteremia, pyelonephritis, bacterial pneumonia, pneumothorax, bleeding from gastric and duodenal ulcers

Abbreviations: ADL, Activities of daily living. MPA, microscopic polyangiitis. NPPV, Non-invasive positive pressure ventilation. HFNC, High-flow nasal cannula. MSSA, Methicillin-Susceptible Staphylococcus Aureus.
*Types of long-term care: Care Level 1: A person faces difficulty in performing essential daily life activities by himself/herself. One person's ability to handle task-based activities in daily life is lower than that of patients in the Support Required 2 category. Care Level 2: The person is in a state similar to that detailed under Care Level 1 but requires more care to be able to perform essential daily life activities. Care Level 3: Compared with the state of Care Level 2, a person's abilities to perform essential daily life activities and task-based activities were significantly lower. Consequently, they require almost constant care. Care Level 4: The person is in a state similar to that detailed in Care Level 3, but his/her ability to act is lower. As a result, they face difficulties in living without constant care. Care Level 5: The person's ability to act is even lower than that of patients in the Care Level 4 category. Consequently, they require constant care.

Discussion

In addition to describing the clinical course of the disease in patients, the cases were classified into three groups based on the primary cause of death: *i*) pneumonia (including COVID-19 pneumonia and aspiration pneumonia), *ii*) exacerbation of preadmission complications, and *iii*) complications that occurred during hospitalization.

Deaths due to pneumonia

In total, 16/20 patients (80%) who died during the delta variant epidemic and 7/24 patients (29%) who died during the omicron outbreak died of pneumonia. The pneumonia group was broadly divided into patients with lesions mainly in the bronchi and surrounding areas and those with lesions mainly in the pulmonary interstitium. In typical viral pneumonia caused by SARS-CoV-2 infection, lesions in the pulmonary interstitium are observed on chest CT scans (*5*). In contrast, in cases where lesions were found in the bronchi and peribronchial centers, the patient likely developed pneumonia secondary to the SARS-CoV-2 infection. The impact of aspiration should also be considered based on the patient's age, ADL, and underlying diseases.

Moreover, 15/20 patients who died during the delta variant epidemic had lesions mainly in the pulmonary interstitium, suggesting that most patients during the delta variant epidemic died due to the progression of viral pneumonia caused by SARS-CoV-2 infection. In animal models, the omicron variant (B.1.1.529) was reported to have similar nasal growth potential as the delta variant (B.1.617.2) but lower growth potential and pathogenicity in the lungs (*6*). In addition, omicron variants replicate rapidly in human airway models and ex vivo cultures of human bronchioles. However, their replication efficiency is reduced in human alveolar models and ex vivo cultures of human lungs (*7*). These findings indicate that omicron variants tend to infect the upper respiratory tract rather than the lungs, which is consistent with the results of previous studies.

Death due to factors other than pneumonia

During the delta epidemic, 4/ 20 patients died from causes other than pneumonia, and during the omicron epidemic, 17/24 patients died from complications before or during hospitalization. In both groups, the deterioration of the general condition due to the SARS-CoV-2 infection may have affected the disease course.

Deaths due to preexisting comorbidities

Among the patients who died from causes other than pneumonia during the omicron variant outbreak, 11 patients (46%) died due to the exacerbation of preexisting comorbidities, representing the highest proportion. These cases involved patients infected with SARS-CoV-2 with severe underlying conditions. The breakdown of these comorbidities included 4 cases of heart failure, 4 cases of malignancy, 1 case of hepatic dysfunction, 1 case of renal dysfunction, and 1 case of extensive cerebral infarction. Worsening underlying conditions, such as cancer and cardiovascular diseases, were considered the primary cause of death. Three deaths were attributed to preexisting comorbidities during the delta variant outbreak: traumatic subdural hematoma, decompensated liver cirrhosis, and acute myocardial infarction.

From the early stages of COVID-19 to the delta variant outbreak, pneumonia accounted for most deaths, necessitating the establishment of facilities capable of managing patients with severe respiratory failure. However, during the omicron variant outbreak, infection control measures must be prioritized for patients with preexisting comorbidities who are at high risk of approaching the end of life before hospital admission.

Deaths due to complications during hospitalization

Factors other than preexisting comorbidities, such as infections, bleeding, and cardiovascular diseases, are the main causes of death among the complications that occur during hospitalization. During the delta variant outbreak, 1 patient died from complications that emerged during hospitalization, and during the omicron variant outbreak, 6 patients died.

Out of the 6 patients who died from complications during the omicron variant outbreak, 4 succumbed to cardiovascular events. The causes of death were hemorrhagic shock due to iliopsoas hematoma, cerebral hemorrhage, aortic dissection, and acute myocardial infarction. The remaining 2 patients died from acute respiratory distress syndrome and septicemia caused by pyelonephritis. Out of these 6 patients, 4 were completely independent in ADL before admission, and 1 required only mild support. No in-hospital deaths were anticipated during admission.

During the omicron variant outbreak, patients with COVID-19 admitted to the hospital who subsequently died showed a notable prevalence of cardiovascular diseases. From a wider standpoint, excess mortality from cardiovascular diseases has been reported domestically and internationally. Nishiura *et al*. (*8*) predicted a significant increase in selective deaths attributed to cardiovascular diseases and senility in Japan during the 2022 fiscal year. Nomura *et al*. (*9*) reported excess mortality due to cardiovascular diseases, respiratory diseases, malignancies, and senility in 2021. Globally, previous reports have indicated a higher incidence of cardiovascular complications in patients with COVID-19 (*10*).

COVID-19 is associated with an increased risk of cardiovascular diseases, potentially leading to

myocarditis, acute coronary syndrome, atherosclerosis, heart failure, thromboembolic events, and arrhythmias. The acute phase of COVID-19 and its long-term effects after acute infection may contribute to increased hospitalization and mortality rates in patients with cardiovascular diseases (*11*). A meta-analysis of 17 cohort studies involving 5815 patients showed that the most common cardiovascular complications are heart failure, myocardial injury, arrhythmias, and acute coronary syndromes (*12*). Even in mild cases, an increased risk of cardiovascular diseases for up to 1 year has been reported, with higher rates of heart failure and stroke (*3*).

The findings of this study corroborate those of previous studies from a broader perspective, indicating the prevalence of cardiovascular complications in COVID-19 cases. However, many aspects of COVID-19 pathogenesis remain unclear. Therefore, data that can contribute to our understanding of the disease and excess mortality can be obtained by collecting epidemiological data on the causes of death, including events with unclear associations with COVID-19. Consequently, starting from April 2023, Japan has revised the case reporting form for COVID-19 Registry Japan (COVIREGI-JP) to collect detailed information on patients who have succumbed to COVID-19, aiming to gather comprehensive data.

In addition to the direct impact on organs caused by COVID-19 infection, observations have pointed to secondary changes, including disruptions in healthcare access due to medical system breakdown and lifestyle alterations, as contributing factors. Brant *et al*. (*13*) reported changes in cardiovascular disease mortality rates in 6 major cities in Brazil. Mortality rates varied, ranging from +46.1% (Manaus) to -7.1% (Rio de Janeiro), with more pronounced effects observed in areas where the healthcare system serving the most socioeconomically disadvantaged populations collapsed. In Brazil's most affected northern region, Jardim *et al*. (*14*) estimated a 2.5-fold excess mortality increase in cardiovascular disease-related mortality in 2020. Several other studies have suggested that barriers to healthcare access contribute to increased cardiovascular diseases (*15, 16*).

Reports on the strain on Japanese medical infrastructure up until the delta variant surge and the fourth wave indicated no overwhelming numbers of COVID-19 cases beyond the capacity of acceptance and no depletion of medical equipment such as ventilators. Thus, it was inferred that the Infection control team capacity was not exceeded. However, during the fourth wave, there were indications of excess mortality for the first time, suggesting a potential reflection of strain on medical institutions. Whether appropriate access to medical facilities is sufficient during the peak of infection spread remains unclear. During the fifth and sixth waves, an increase in difficult cases of emergency transport was noted alongside a rise in the number of infected patients, pointing to the possibility of strain on medical infrastructure (*17*).

The limitations of this study were as follows. The analysis was limited to hospitalized patients from a single facility in Japan, making it difficult to directly extrapolate the results to the causes and backgrounds of COVID-19-related deaths in all patients. As we did not observe the causes and backgrounds of the patients who received at-home treatment, their characteristics may differ from those of hospitalized patients. Therefore, we plan to conduct future observational studies involving multiple facilities. Additionally, we did not conduct a comparison with surviving patients, and the causal relationship between patient background and cause of death remains unclear. Furthermore, as the period following the omicron variant outbreak is expected to show continued circulation of different variants, the trends observed in this study may change under the influence of different variants.

In conclusion, from the early stages of COVID-19 to the delta variant outbreak, numerous cases of severe respiratory failure caused by the typical SARS-CoV-2 infection leading to viral pneumonia were reported. Establishing medical systems capable of treating patients with severe respiratory failure was crucial during this period. However, during the omicron variant outbreak, approximately two-thirds of the deceased patients at our hospital had non-pneumonia-related conditions as the primary cause of death, exhibiting a different clinical profile compared with the delta variant cases.

Moving forward, we aim to adapt our medical systems by considering these changing patient profiles and continue to collect data at the macro level. We hope that this study, which observed changes in the number of deceased patients between delta and omicron variant outbreaks, will be the starting point for further understanding of the pathogenesis of the disease.

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BRIEF REPORT

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The potential association between COVID-19 and Parkinson's disease-like symptoms

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Abstract: Although the initial triggers of Parkinson's disease (PD) remain largely unknown, viral infections have been implicated in some cases. In this study, we investigated the neurological sequelae and explored the potential association between post COVID-19 conditions and the development of Parkinson's disease-like symptoms by conducting a questionnaire survey on patients with COVID-19. We surveyed patients who had recovered from COVID-19 and visited the outpatient service of the institution from February 2020 to November 2021. Demographic and clinical data and data regarding the presence and duration of post COVID-19 conditions were obtained. The most common symptom was insomnia, experienced by 141 participants (27.9%). Bradykinesia was the next most common symptom, experienced by 66 participants (13.1%). Moreover, 18 (3.6%) participants reported persistence for > 180 days. Notably, the median duration of bradykinesia was 90 days. The proportions of patients with dysbasia, resting tremor, not previously noted constipation, and somniloquy were 9.1%, 5.0%, 4.8%, and 2.8%, respectively. Few participants had any of these symptoms lasting for more than 180 days. None of the participants was first diagnosed with PD, other neurological diseases, or dementia after COVID-19. Longitudinal follow-up surveys are needed to better understand the relationship between COVID-19 and PD.

***Keywords*:** Parkinson's disease (PD), neurological Sequelae, post COVID-19 Conditions

Introduction

The Coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread worldwide since early 2020 (*1*). Approximately 10–20% of people experience various mid- and long-term effects after recovering from COVID-19 (*2*). Notably, the sequelae of COVID-19 are known as post COVID-19 conditions. Frequent symptoms of post COVID-19 conditions include fatigue, cough, and dysosmia; however, multiple symptoms often overlap and persist (*3-6*). Furthermore, post COVID-19 is known to affect neurological and motor functions, and myalgic encephalomyelitis/chronic fatigue syndrome occurs in the nervous system after recovery from COVID-19 (*7*). A recent study demonstrated that SARS-CoV-2 infection may lead to cognitive decline and brain atrophy including olfactory pathways (*8*), further highlighting the association between COVID-19 and neurodegenerative disorders.

Parkinson's disease (PD), a progressive neurodegenerative disorder, is characterized by a range of motor and non-motor symptoms. Importantly, years or even decades before the onset of the typical motor symptoms, the PD process is associated with a characteristic prodrome (*9*). Although the initial triggers of PD are largely unknown, viral infections have been implicated in some cases (*10*). Notably, patients developed clinical parkinsonism a few weeks after contracting COVID-19 (*11-13*).

One of the well-known sequelae of COVID-19 is dysosmia, which is an early symptom in the onset of PD (*14*). We hypothesized that COVID-19 might trigger the onset of PD. In this study, we investigated the neurological sequelae and the potential association between post COVID-19 conditions and development of Parkinson's disease-like symptoms by conducting a questionnaire survey on patients with COVID-19.

Patients and Methods

This study was designed as a single-center, cross-sectional survey in which a self-reported, online, or paper-based questionnaire was sent to eligible patients in February 2022 without reminders (*4*). Participation in this survey was voluntary, but not anonymous. Participants who recovered from COVID-19 were requested to complete and return the questionnaire. Informed consent was obtained through a consent checkbox,

either online or *via* a paper-based questionnaire. The study was reviewed and approved by the institutional ethics committee, specifically the Ethics Committee of National Center for Global Health and Medicine (NCGM) (NCGM-S-004406-00). Furthermore, the study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients who recovered from COVID-19 and visited the outpatient service of the institution between February 2020 and November 2021 to undergo a pre-donation screening test for COVID-19 convalescent plasmapheresis were recruited (*15*). Most participants had received acute-phase treatment for COVID-19 in other hospitals. Furthermore, all participants in this study were Japanese, as the screening test was specifically designed for Japanese patients.

We developed a questionnaire based on findings from our previous study on prolonged- and late-onset symptoms of COVID-19 and comprehensive discussions among the authors. Moreover, we attempted to minimize the number of questions required to maximize the response rate. Five nonmedical employees of NCGM were included in the pilot study and provided feedback on the items' content, clarity, and format and whether the survey questions were self-explanatory. Minor revisions were made in response to the feedback.

Patient characteristics were investigated, including information regarding the acute phase of COVID-19 and the presence and duration of symptoms related to COVID-19. Disease severity was categorized as follows: mild, requiring no oxygen therapy; moderate, necessitating oxygen therapy but not mechanical ventilation; and severe, involving mechanical ventilation with or without extracorporeal membrane oxygenation. Post-acute phase symptoms related to PD included insomnia, somniloquy (Rapid eye movement sleep behavior disorder [RBD] like symptoms), resting tremors, bradykinesia, dysbasia, and not previously noted constipation. This information was obtained using an online/paper-based questionnaire, as it was difficult to obtain this information from medical records, given that many participants in this study were treated for the acute phase of COVID-19 at other hospitals.

Patient characteristics, the presence of pneumonia, disease severity, and treatments administered during the acute phase of COVID-19 were described using the median and interquartile range for continuous variables and as absolute values (*n*) accompanied by percentages (%) for categorical variables.

The proportions of patients with prolonged symptoms, those with symptoms lasting for at least 30 days within 180 days of symptom onset, and those with symptoms lasting beyond 180 days were described.

Results and Discussion

A total of 505 responses were obtained. All patients were diagnosed with COVID-19 between February 2020 and December 2021. The median number of days (interquartile range) from COVID-19 diagnosis to completion of the questionnaire survey was 392 (243–473 days). Moreover, the median age of the participants was 48 years, and 59.6% were women. Notably, all participants were Japanese. In terms of disease severity, 393 (77.8%), 58 (11.5%), and 5 (1.0%) participants had mild, moderate, and severe disease, respectively. Furthermore, 59 (11.7%) patients received corticosteroids, and 57 (11.3%) received antivirals. A total of 237 (46.9%) participants had no underlying medical conditions. Only one participant reported an underlying neurological disease (Table 1).

The most common symptom was insomnia, experienced by 141 participants (27.9%). However, none of the participants reported that it persisted for more than 180 days, with a median duration of insomnia of 45 days. Bradykinesia was the next most common symptom, experienced by 66 participants (13.1%). Additionally, 18 (3.6%) patients reported persistence for > 180 days; the median duration of bradykinesia was 90 days. The proportions of patients

Table 1. Demographic and clinical characteristics of the participants (*n* = 505)

Characteristics	Value
Age, median (IQR), years	48.0 (42.0, 55.0)
Female sex, *n* (%)	301 (59.6)
Ethnicity, *n* (%)	
Japanese	505 (100)
Timing of the interview	
Days since symptom diagnosis of COVID-19, median (IQR)	392 (243, 473)
Individual comorbidity, *n* (%)	
No underlying medical conditions	237 (46.9)
Hypertension	70 (14.9)
Bronchial asthma	69 (14.7)
Dyslipidemia	59 (12.6)
Diabetes	19 (4.1)
Malignancy	13 (2.6)
Connective tissue disease	4 (0.8)
Chronic kidney disease	3 (0.6)
Immunodeficiency	2 (0.4)
Neurological disease	1 (0.2)
Highest severity during clinical course of COVID-19	
Mild	393 (77.8)
Moderate	58 (11.5)
Severe	5 (1.0)
Unknown	49 (9.7)
Pharmacological treatments	
Antiviral	57 (11.3)
Corticosteroids	59 (11.7)
Casirivimab/ imdevimab	4 (0.8)
Sotrovimab	4 (0.8)

with dysbasia, resting tremor, not previously noted constipation, and somniloquy were 9.1%, 5.0%, 4.8%, and 2.8%, respectively. Notably, few participants had any of these symptoms lasting for more than 180 days. Finally, none of the participants was first diagnosed with PD, other neurological diseases, or dementia after COVID-19 (Table 2).

Direct tissue damage by SARS-CoV-2 infection, constitutive inflammatory responses including autoimmunity, and psychiatric impairment have been suggested as underlying etiologies of long COVID-19 (*16*). Furthermore, it has already been reported that COVID-19 exacerbates motor function and neurological deficits in existing patients with PD and causes new symptoms (*17*). Specifically, it is thought that the insufficient response to dopaminergic drugs after infection altered transport of dopaminergic drugs through the blood-brain barrier (BBB) after systemic infection, and altered dopamine metabolism and receptor signaling are due to the response to proinflammatory cytokines (*18*). Another possibility is that extracerebral inflammatory processes can exacerbate pre-existing neuroinflammatory processes (*19*). Moreover, COVID-19 may indirectly influence symptoms in PD through factors such as substantial changes in routine life, social isolation, stress, anxiety, and prolonged immobility (*20*). However, the causal relationship between COVID-19 and the onset of PD and the mechanism by which COVID-19 triggers neurodegeneration remain determined (*18*). Importantly, studies on the risk of developing PD have shown that patients with viral or bacterial infections have a 20% higher risk of developing PD than controls do (*21*), and COVID-19 may be involved in developing PD.

SARS-CoV-2 enters the brain through the olfactory bulb, axonal transport from peripheral nerves, and hematogenous pathways through the BBB (*22*). Post-infection, immune-mediated processes play an important role in the development of PD (*23*). Interestingly, 13.1% of the participants in this study reported bradykinesia, and 3.6% reported that their symptoms persisted for more than 180 days. Bradykinesia emerged as a distinct long-term sequela, in contrast to other items in the study, which few participants reported as persisting beyond 180 days. While the direct relationship between bradykinesia and early symptoms of PD remains unclear, such manifestation may be a transient symptom of disuse and muscle weakness linked to COVID-19. Notably, all participants who reported dysbasia improved within the 180-day timeframe. Therefore, bradykinesia is considered a significant symptom of post-COVID-19, and continued follow-up is recommended.

Olfactory dysfunction is an early and sensitive marker of the preclinical phase of PD (*14*). α-synuclein pathology in PD is thought to originate from enteric or peripheral autonomic nervous system, or brain regions such as olfactory bulb and amygdala (*24*). In a prior study, 57.8% of COVID-19 patients reported olfactory disturbances; notably, 20.1% of these persisted for several months, and 10.3% continued for more than a year. Although olfactory disturbances are a frequent sequela of COVID-19, none of the patients in this study developed PD after contracting COVID-19. One reason is that this study's median period from onset to investigation was as short as 392 days, which was not long enough to lead to PD onset. Notably, previous studies examining the association between viral infections and the development of PD have reported that a period of 5–10 years after infection is associated with the development of PD (*25*).

This study had some limitations. First, there may be recall bias because the questionnaire survey was conducted on subjects who had been infected with COVID-19 for some time. Second, the participants may have reported resting tremors that they confused with COVID-19 chills. Third, difficulties in walking and movement may be caused by disuse after contracting COVID-19. Fourth, the period from the onset of COVID-19 to the investigation was short, and the time to the onset of PD may have been too short. COVID-19 is a disease with rising prevalence globally. It should also be noted that the questionnaire survey used in this study, which asked about subjective symptoms, is not a diagnostic criterion for PD on its own. The Japanese Ministry of Health, Labour and Welfare defines

Table 2. Number of participants with post-acute COVID-19 symptoms and the persistence of symptoms

Number of patients with the symptom (%)	Number of patients with the symptom (%)	Lasting less than 30 days (%)	Lasting at least 30 days within 180 days since the onset (%)	Lasting more than 180 days (%)	Duration of symptoms in days (median, IQR)
Insomnia	141 (27.9)	115 (22.8)	10 (2.0)	0 (0)	45 (10,255)
Bradykinesia	66 (13.1)	22 (4.4)	21 (4.2)	18 (3.6)	90 (30,255)
Dysbasia	46 (9.1)	40 (7.9)	4 (0.8)	0 (0)	90 (14,240)
Rest tremor	25 (5.0)	22 (4.4)	2 (0.4)	0 (0)	30 (9, 90)
Not previously noted constipation	24 (4.8)	17 (3.4)	4 (0.8)	1 (0.2)	30 (14,270)
Somniloquy	14 (2.8)	10 (2.0)	1 (0.2)	0 (0)	7 (7, 180)
First diagnosed with Parkinson's disease or other neurological disease or dementia	0 (0.0)	-	-	-	-

diagnostic criteria include the presence of parkinsonism, absence of specific abnormalities on the head computed tomography or magnetic resonance imaging, absence of exposure to drugs or toxic substances that cause parkinsonism, and improvement of parkinsonism with antiparkinsonian drugs (*26*). Dopamine transporter positron emission tomography (PET) data or dopamine receptor PET data, and a more commonly used tool such as the unified parkinson's disease rating scale is also useful for diagnosis. Therefore, it is necessary to conduct a follow-up survey to determine whether the number of patients with PD will increase.

Conclusion

In this study, none of the patients developed PD after COVID-19 infection. Long-term follow-up with more patients may clarify the relationship between COVID-19 and PD. Therefore, longitudinal follow-up surveys are warranted to better understand the relationship between COVID-19 and PD.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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BRIEF REPORT

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The role of endocrine gland derived vascular growth factor/ Prokineticin-1 in human prostate cells

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Abstract: In steroidogenic tissues, a novel class of angiogenic molecules known as endocrine gland-derived vascular endothelial growth factors (EG-VEGF)/prokineticins are primarily produced. Here, we investigated how EG-VEGF/PROK1, a member of PROKs family, and its receptor are able to affect cellular motility in both non-neoplastic and cancerous human prostate cells. Using Western blot and motility test studies, EPN cells, a non-transformed cell line and Cancer Epithelial Prostatic Cells (CEPC) were employed as cellular models in the current investigation. Western blot examination of EPN normal prostate cells treated with EG-VEGF/PROK1 revealed that ERK1/2 was rapidly phosphorylated within 5, 10, and 20 minutes, while CEPC had high and sustained ERK1/2 activity at the same periods. Then, compared to normal EPN prostate cells, CEPC treated with EG-VEGF/PROK1 for up to 72 hours demonstrated enhanced cell motility. Based on our findings, EG-VEGF/PROK1 may play a role in prostate cancer progression by controlling angiogenesis and the motility of metastatic cells in CEPC cells, likely as a consequence of ERK1/2 activation, as contrasted to EPN normal prostate cells.

***Keywords*:** prostate cancer, EG-VEGF, EPN, CEPC, ERK1/2

Introduction

Angiogenesis plays a crucial role in the development, invasion, and metastasis of various cancers, including prostate cancer (*1,2*). Creating new microvascular endothelium is crucial for optimal prostate development, in fact (*3,4*). A link between the hormonal regulation of prostate tissue and angiogenesis caused by the paracrine actions of endothelial cells has been proposed. Prostate cells generate a number of angiogenic substances, including vascular endothelial growth factor (VEGF). The majority of prostate cancers have been shown to have higher VEGF levels and microvessel density, both of which are associated with a worse prognosis (*4-8*). Two novel endocrine gland-derived vascular angiogenic factors (EG-VEGF)/prokineticin-1 (PROK1) and prokineticin-2 (PROK2) that specifically affect endothelial cells (EC), have recently been identified (*9*). PROK1 is similar to Prok1, a previously cloned mammalian ortholog of mamba intestinal toxin-1(MIT-1) (*10*). However, other EC types, such as those derived from the aorta, umbilical vein, and dermis, are not affected by PROK1 and PROK2, which induce proliferation and migration of EC derived from adrenal capillaries (*11-13*). These peptides, which are structurally unrelated to VEGF and have 10 cysteine residues in the same places in all family members, control a variety of biological processes in steroidogenic and non-steroidogenic tissues, including smooth muscle contraction and, in particular, angiogenesis.

G-protein-coupled receptors recently discovered and given the names PROK-R1 and PROK-R2 (*11-13*) carry out the physiological actions of PROK1 and PROK2. Recombinant PROKs in nanomolar concentrations bind to and activate these receptors. In accordance with the effects of PROKs on smooth muscle contraction and angiogenesis (*11-13*), activation of PROK-R causes calcium mobilization, stimulation of phosphoinositide turnover, and activation of ERK1/2 pathways. The overexpression of PROK1 in a colorectal cancer cell line has recently been shown to cause angiogenesis and tumor growth after injection in a nude mouse model (*14*). Contrarily, PROK1 expression was diminished in advanced ovarian cancer but was still seen in the early stages of this illness (*15*), but its expression was typically undetectable in endometrial carcinoma (*16,17*). In order to assess the possible contribution of this factor to human prostate cancer, we examined the effects of PROK1 on ERK1/2 activation and cellular motility in normal and malignant human prostate cells.

Materials and Methods

Prostate cell lines and primary prostate cell cultures

Different centrifugations of minced and prostatic tissues treated with collagenase (Collagenase IV, Gibco-BRL, Milan, Italy, 10 mg/mL) separated cancer epithelial prostatic cells (CEPC). The CEPC were plated on keratinocyte-SFM (KFSM) medium from Gibco-BRL in Milan, Italy, which also included 5% fetal bovine serum (FBS), bovine pituitary extract (10 mg/mL), epidermal growth factor (10 ng/mL), cholera toxin (10 ng/mL), and antibiotics (fungizone and penicillin-streptomycin). After EDTA-trypsin treatment, cultures were separated and utilized at first passage at confluence. According to previously documented techniques (*18,19*), the epithelial type was determined if cytokeratin immunostaining was positive in almost all of the cells. The high expression of the proliferative antigen Ki67 and, more specifically, the high expression of mutated p53 protein, as shown by immunoreactivity with the monoclonal antibodies clone Ki-67 (DAKO, Milan, Italy) and Pab 240 (Serotec, Delta Biological, Italy), respectively, confirmed the malignant nature of cells derived from prostate carcinomas. The experimental methods were performed at least three times with four cell strains taken from CEPC. Our group (*19*) identified and characterized a non-transformed cell line known as EPN cells, which were grown in HAM-F12 enriched with 3% FBS and antibiotics (fungizone and penicillin-streptomycin). Every culture was kept alive at 37°C in a humidified 5% CO_2 environment.

Western blot analysis and protein extraction

Samples of prostate cells were homogenized directly into the lysis buffer, which contains the following ingredients: 50 mM HEPES, 150 mM NaCL, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton-X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mg aprotinin, 0.5 mM sodium orthovanadate, and 20 mM sodium pyrophosphate. Centrifugation was used to clarify the lysates at a rate of 14,000 × 10 min. The amount of protein was determined using a Bio-Rad test (Bio-Rad, München, Germany), and it was then boiled for five minutes in Laemmli buffer (Tris-HCl pH 6.8, 0.125 M, SDS 4%, glycerol 20%, 2-mercaptoethanol 10%, and bromophenol blue 0.002%), as previously reported (*19-25*). Proteins were exposed to reducing conditions SDS-PAGE (15% polyacrylamide). Using pre-stained protein standards, the full transfer of proteins to nitrocellulose membranes (Immobilon Millipore Corporation) during electrophoresis was determined (Bio-Rad, Hercules, CA). The membrane was incubated with the primary antibody against ERK1 (1:1,000; #sc-94-G, Santa Cruz Biotechnology Inc., Santa Cruz, CA) and P-ERK1 for 1 h after blocking with TBS-BSA (25 mM Tris, pH 7.4, 200 mM NaCL, 5% bovine serum albumin), at room temperature. After 45 minutes at room temperature, membranes were treated with the horseradish peroxidase-conjugated secondary antibody (1:4,000), and an ECL system was used to monitor the response (Amersham Life Science, UK).

Wound recovery

Confluent cells were cultivated in KSFM containing 3% FCS before being maintained for 48 hours in serum-free media. In order to lower the content of endogenous steroids, the monolayers were scraped with a sterile, disposable 200 µL plastic pipette tip and then treated with KSFM, KSFM added with 5% charcoal-treated FCS (5% DCC), or 3% FCS. Then, monolayers were captured at various time frames *via* photography (24 h). The shown figure is representative of three independent assays.

PCR

As previously disclosed (*26*), reverse transcriptase-polymerase chain reaction (RT-PCR) RNAs were reverse transcribed using 5 µg of total RNA (*25*). We performed an RNA transcription without the use of reverse transcriptase to generate a negative control for the amplification reactions. A total of 50 µL of cDNA (400 ng) amplified using RT of RNAs was mixed with 10 mmol Tris-HCl, 1.5 mmol MgCl2, 50 mmol KCl (pH 8.3), and 100 ng 5'-3' end primers. 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 90 sec each made up the PCR conditions. In a semiquantitative PCR, these genes were amplified using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (22 cycles) as previously reported to assess the variability in the expression of PROK1 and PROK-R1 (*25*). For GAPDH, we employed oligonucleotide sequences (*25*). Additionally, we utilized the following primers: PROK-R1 sense (5'-GCG GCA TTG GAA ACT TCA-3') and antisense (5'-GGC CCA CGA ATT CTA TGC C-3') for PROK-R1and PROK1 sense (5'-CGC GAG TCT CAA TCA TGC TCC T-3') and PROK1 antisense (5'-GGC AAG GCG CTA AAA ATT GAT G-3'). After that, PCR products were separated using a 100-bp DNA ladder from Life Technologies as a size marker on a 1.2% agarose gel that also included ethidium bromide.

Statistic evaluation

The results, which came from at least three different experiments with each point carried out in triplicate, were presented as mean + SE (standard error of the mean). The analysis of variance was used to compare the means.

Results and Discussion

Both non-neoplastic and cancerous epithelial prostate cells express PROK1 and PROK-R1. In order to determine how these genes are expressed in CEPC primary cultures and the EPN cell line, we used semiquantitative RT-PCR. EPN and CEPC both contained the mRNAs for PROK1 and PROK-R1 (Figure 1A).

Cultures of EPN and malignant (CEPC) human prostate cells, fasted for 48 h and maintained in 0.5% FCS, were treated with 40 nM PROK1 to see whether it activated the ERK1/2 pathway. Then, in cell lysates made after 5, 10, and 20 min of treatment, Western blot analyses were performed to confirm that ERK1/2 was activated. Figure 1B demonstrates that within 5 min, PROK1 produced a fast phosphorylation of Erk1/2 in EPN cells, which persisted for up to 20 min. After 5 minutes, PROK1 also significantly enhanced ERK1/2 phosphorylation in CEPC cultures, however unlike in normal cells, the elevated phosphorylation levels were greater than in the normal EPN cell line (Figure 1C). Since there was no change in the ERK1/2 protein, staining with anti-Erk1/2 and anti-phospho-Thr/Tyr antibodies demonstrated that the action of PROK1 on ERK1/2 was caused by phosphorylation of the enzyme rather than an increase in ERK1/2 expression (Figure 1C).

We examined the role of PROK1 in the control of prostate epithelial cells' motility using a wound-healing experiment in EPN and CEPC cells. Before being scraped and thereafter being stimulated with FCS, confluent cell monolayers were kept in serum-free media for 48 hours. Cell motility in CEPC cells was higher than in EPN cells after 15 hours of serum administration. Contrarily, when cells were kept in serum-free media, neither EPN nor CEPC showed much evidence of cell motility (Figure 1, D and E). Contrary to what may be expected, the scrape wound persisted in the monolayer of EPN cells for 24 hours after PROK1 (40 nM) was added to the CEPC cells (Figure 1, D and E). Few CEPC cells began to migrate from the wound's edges in the absence of serum, while EPN cells remained stationary.

Earlier, we reported that whereas PROK1 expression is scarcely perceptible in the non-neoplastic human prostate, it is significantly increased in prostate cancer. We specifically demonstrated the expression of PROK1 by prostate epithelial cells and the association between expression levels and Gleason scores (*25*). Additionally, we discovered that the amount of the PROK1 protein increased significantly and steadily as the grade of the prostate cancer increased from low to medium to high, showing the significance and specificity of PROKs as predictive biomarkers for prostate cancer progression (*25*). Further evidence that PROKs may directly affect epithelial cells comes from the finding of EG-VEGF receptor (PROK-R1) transcripts in both normal (EPN)

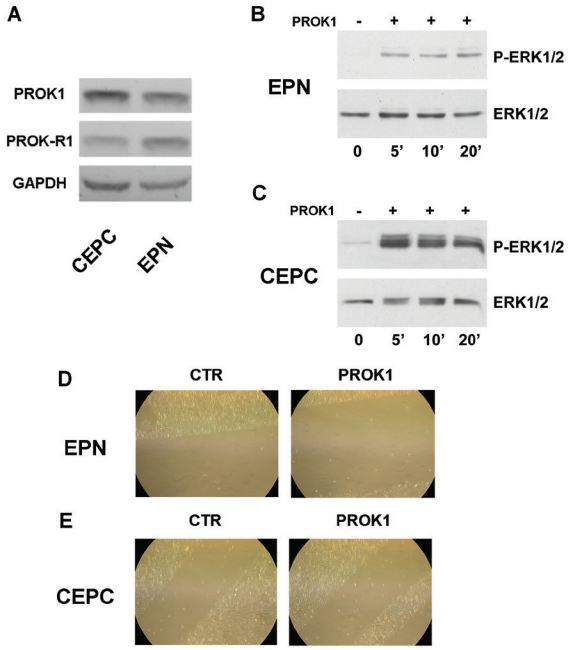

Figure 1. PROK1 activity in prostate-derived cell models. (A) GAPDH, PROK1, and PROK-R1 primers were used to reverse transcribe and amplify total RNA, respectively. PCR results were run through a 1.2% agarose gel electrophoresis, stained with ethidium bromide, and then captured on camera. Using GAPDH as an internal reference, semiquantitative RT-PCR was used to ascertain the mRNA levels of PROK1, PROK-R1. PCR results were run through a 1.2% agarose gel electrophoresis, stained with ethidium bromide, and then captured on camera. 40 nM PROK1 was applied to EPN **(B)** and CEPC **(C)** for the specified durations. Lower panels of (B) and **(C)** show blots with antibodies against Erk1/2 protein, whereas the top panels of **(B)** and **(C)** display blots treated with antibodies raised against phospho-Erk1/2. There are two distinct bands at 44 and 42 kDa. Three different experiments are shown by the blots. EPN **(D)** and CEPC **(E)** were damaged while serum-starved, cultured in KSFM (SF), and KSFM was subsequently supplemented with 40 nM of PROK1. After 24 hours of therapy, the morphology of cell motility is shown.

and cancerous (CEPC) prostate cells (*25*). The biological foundation of tumor angiogenesis has long piqued researchers' curiosity in cancer. Blood vessel changes are associated with abnormal pathways, apoptosis, androgen receptor signalling, signal transduction, cytokines, and cell adhesion molecules. The VEGF pathway is one of the main regulators of this process. This element, which is widely expressed in both non-neoplastic and cancerous tissues, is primarily accountable for the formation and upkeep of the aberrant tumor vascular network. Current preclinical and clinical investigations in cancer on the suppression of the VEGF pathway were inspired by these findings. Anti-VEGF medication has extra systemic consequences, such as causing endothelial cell death and altering the functioning of the vascular bed, despite the fact that it may have antiangiogenic qualities and seem promising. LeCouter *et al*. discovery's of the EG-VEGF gene, a novel angiogenic agent mostly produced in endocrine cells that specifically works on the endothelium of endocrine gland cells, has opened up

new perspectives on the pathogenesis and treatment of endocrine-related tumors (*9*). In fact, the discovery of angiogenic tissue-selective factors may open the door to tissue-specific angiogenic treatments with minimal systemic side effects. The fundamental reason for the divergence between EG-VEGF and VEGF is the selectivity of expression region. In actuality, PROK1 is only present in cells that produce hormones, such as those in the ovaries, testes, and adrenal gland (*9,27-29*). About how PROKs are expressed in cancerous tissues, not much is known. Recent research showed that colorectal cancer cells express PROK1. Antisense PROK1 injections into mice also resulted in angiogenesis and tumor development suppression. Prostate cancer and PROKs have not been linked, and nothing is known about the expression of PROK-R in non-neoplastic and cancerous human prostate (*14,15*).

In the current work, we demonstrate that the malignant epithelial prostate cells (CEPC) and EPN, a human prostate epithelial cell that has not undergone transformation, have distinct levels of MAP kinase (ERK1/2) activity. This may be partially explained by our recent discovery that normal and cancerous human epithelial prostate cells exhibit varying degrees of fast, non-genomic effects of PROK1 on Erk1/2 (*30*). We demonstrate that a 5-minute PROK1 therapy causes the Erk1/2 pathway to be activated in both CEPC and EPN. Erk1/2 phosphorylation levels being altered suggests that regulatory kinases and/or phosphatases upstream and/or downstream of Erk1/2 are altered in tumor cells because of the pathway's rapid stimulation, which is comparable to many extracellular signals that start cytoplasmic signal transduction pathways. Actually, multiple cancer processes have been altered, resulting in altered cell phenotypes that affect the regulation of cell proliferation. Migration of tumor cells to distant organs is not a routine process. Breast, prostate, and lung cancers – the most prevalent solid tumors – metastasize particularly to the bones (*30*). The preferred spreading of certain tumors to the bones is determined by the biological characteristics of the cancer cells and the environment of the metastatic target site (*30*).

Cell motility is a crucial element in the spread of prostate cancer. Our findings suggest that PROK1 may be a novel potential component involved in the stimulation of cell migration and motility. Finding the genes and related molecular mechanisms that cause epithelial prostate cells to transdifferentiate into a transformed phenotype is essential for developing novel prostate cancer treatment methods. Therefore, the presence or absence of molecules associated to motility may be exploited to help with the diagnosis and prognosis of human prostate cancer. Modified expression of these genes may govern the metastatic potential of any specific prostate tumor. Our study demonstrates that PROK1 has stimulating effects on prostate epithelial tumor cell growth and migration *in vitro*, suggesting a role in the neoplastic progression.

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Do hilar clamping and renorrhaphy influence postoperative renal function after partial nephrectomy?

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Abstract: Preservation of renal function is an important goal of partial nephrectomy (PN) for renal tumors. Several attempts to preserve postoperative renal function, including hilar control surgery and omission of renal cortical renorrhaphy, have been reported, but the influence of each procedure remains controversial. We conducted a literature review based on PubMed to summarize the current situation and clarify the influence of each procedure on postoperative renal function. Effects of hilar control, omitting renorrhaphy, and a combination of both on post-PN renal function were reviewed. While hilar clamping does not influence postoperative renal function, cortical renorrhaphy tends to deteriorate. Parenchymal ischemia/reperfusion by hilar clamping leads to acute kidney injury through production of radical oxygen species. Recent randomized controlled studies, however, showed no differences in the postoperative renal function between on- and off-clamp laparoscopic PN. Finally, the effects of soft coagulation on renal parenchymal denaturation and postoperative renal function were reviewed. Although soft coagulation can lead to denaturation and necrosis of the renal parenchyma, the shortened warm ischemic time might positively affect postoperative renal function. In conclusion, off-clamp, non-renorrhaphy PN is feasible and safe for small renal tumors. Renorrhaphy, but not hilar clamping, tends to worsen postoperative renal function.

***Keywords*:** estimated glomerular filtration rate, kidney failure, nephrectomy, organ preservation

Introduction

Radical nephrectomy was the standard treatment for renal tumors until Novick *et al*. pioneered partial nephrectomy (PN) in the early 1980s (*1-3*). In the beginning, PN was applied under absolute indication for tumors in solitary kidneys, bilateral renal tumors, and patients with impaired renal function. Subsequently, the surgical technique was refined, and the surgical indication continued to expand gradually to selective patients.

The preservation of renal function is an important goal in the treatment of renal tumors and PN. A negative surgical margin, warm ischemia time less than 25 min, and no urological complications are the trifecta of PN (*4*). Furthermore, pentafecta is defined as the achievement of a trifecta with the addition of preserving over 90% estimated glomerular filtration rate (eGFR) and no chronic kidney disease stage upgrading after 1 year (*5,6*). Several methods have been introduced to preserve renal function, including omitting renal hilum clamping, renal cortical renorrhaphy, or a combination of both. The influence of each technique on postoperative renal function remains unclear. Herein, we review the current evidence regarding the influence of renal pedicle clamping and renorrhaphy on postoperative renal function. PubMed was searched to identify relevant articles published up to January 15, 2023. Detailed information is provided in Table 1.

Renal hilum clamping

Hilar control techniques, including off-clamp, selective/super-selective clamp, or early unclamp surgeries, may contribute to reduced renal parenchymal ischemia and better preservation of postoperative renal function. There are multiple reports on the pros and cons of the effect of hilar control on postoperative renal function (*7-18*). Regarding surgical complications, one multicenter propensity score-matched case-control study concluded that off-clamp robot-assisted PN (RAPN) is feasible for a small subgroup of renal tumors without postoperative complications, although off-clamp surgeries are at an increased cost of higher estimated blood loss and conversion to radical nephrectomy (*18*).

As for postoperative renal function, parenchymal ischemia/reperfusion by hilar clamping lead to acute kidney injury through production of radical oxygen species (*19*). A meta-analysis reported that short- and long-term renal function are superior in the hilar

Table 1. Search strategy summary

Items	Specification
Date of search	January 10, 2023
Databases and other sources searched	PubMed
Search terms used	Hilar clamping, kidney function, off-clamp, partial nephrectomy, and renorrhaphy
Timeframe	Not applicable
Inclusion and exclusion criteria	Inclusion criteria: *i*) The type of literature should be either a prospective study, a retrospective study, or a meta-analysis; *ii*) The literature focus on the maintenance of perioperative renal function; *iii*) The research subjects must meet the criteria for undergoing partial nephrectomy as outlined in the guidelines; *iv*) Only documents published in English were considered. Any studies that do not meet one or more of these inclusion criteria were excluded.
Selection process	A systematic search was conducted on 10/1/2023 using PubMed with the keywords listed above. The relevant search results were selected for this narrative review.
Any additional consideration	Not applicable

control surgery groups to hilar clamping surgery groups (*20*). After that report, however, results of two prospective randomized control trials, the EMERALD (NCT03679572), and the CLOCK (NCT022/7987) have been published with contrary results (*21,22*). First, the EMERALD study compared the six postoperative month eGFR changes in the operated kidney after RAPN with super-selective clamping and early artery unclamping. The relative eGFR reduction in the operated kidney were not significantly different (-21.4% *vs.* -23.4%, $p = 0.7$) (*21*). Considering the absence of trend in favor of super-selective surgery, the study was interrupted before the entry reached the originally designed number. The CLOCK II prospective randomized study compared effects of on-clamp and off-clamp surgery on postoperative renal function. In this study, 69 of 164 patients (42%) assigned in off-clamp group underwent on-clamp surgery, while 23 of 160 patients (14%) in on-clamp group underwent off-clamp surgery due to tumor complexity and surgeons' preference. They showed no differences in the eGFR between on- and off-clamp laparoscopic PN within 24 months of operation both in intention-to-treat analysis and per-protocol analysis (*22*). Absolute variation in eGFR at 6 months was -6.8 mL/min and -4.2 mL/min for on- and off-clamp RAPN, respectively (*22*). Complication rates were similar between groups (*23*). Taken together, hilar control surgery is feasible and safe for small renal tumors, while its contribution to postoperative renal function is practically small.

Renorrhaphy

Renorrhaphy was first introduced in partial nephrectomy to minimize postoperative complications by hemostasis and closure of the collecting system. In association with preserving parenchyma, necessity of renorrhaphy has been an issue to be discussed. Considering the risk of damaging renal vessels and increasing warm ischemic time that result in reducing renal parenchyma, growing application of non-renorrhaphy technique have been observed (*24-29*).

A meta-analysis registered in the PROSPERO study (CRD42022293977) analyzed 634 patients from 5 retrospective studies. The results showed a significant benefit of the non-renorrhaphy technique in terms of operating and warm ischemic time and, thus, preservation of renal function, compared with that by the renorrhaphy technique. The weighted mean difference for eGFR decline was -4.19 mL/min with a 95% confidence interval of -7.64 to -0.73 ($p < 0.001$). However, they found no difference in postoperative complications between the groups (*30*).

Renorrhaphy is divided into two parts: medullary and cortical layers, also known as inner and outer layers, respectively. Hence, some comparative studies compare single- and double-layer renorrhaphy (both medullary and cortical layer renorrhaphy) (*29,31,32*). Another meta-analysis analyzing single- versus double-layer renorrhaphy showed a benefit of the single-layer technique in the preservation of postoperative renal function (-3.19 mL/min *vs.* -6.07 mL/min, $p = 0.01$) (*33*). The difference could partly be explained by damage to parenchymal vessels, shortening of the warm ischemic time, and reduction in renal parenchyma. In this regard, the results of an ongoing randomized prospective study (NCT02131376) whose endpoint includes the impact of cortical renorrhaphy on renal volume loss and postoperative renal function are awaited.

Therefore, non-renorrhaphy surgery might contribute to the preservation of postoperative renal function by avoiding damage to renal vessels, shortening the warm ischemic time, and preserving renal parenchymal volume.

Off-clamp, non-renorrhaphy PN with a new hemostasis technology

Considering the effects of hilar clamping and renorrhaphy described above, the omission of both is an inevitable attempt. However, owing to the difficulty in controlling bleeding during tumor resection, the surgical indication should be strictly limited. For instance, predominantly exophytic tumors less than 4 cm in diameter are good candidates for off-clamp, non-renorrhaphy surgery. Although the safety and feasibility of this technique have been reported, comparative studies on off-clamp, non-renorrhaphy PN in laparoscopic settings are lacking *(34-36*).

We recently reported the surgical results of off-clamp, non-renorrhaphy open PN performed in a single institution (*37,38*). In our study, hemostasis was performed using a monopolar SOFT COAG system (VIO300D, ERBE, Germany). Medullary renorrhaphies were performed using 4-0 VICRYL® only when the collecting system was opened. The mean eGFR preservation at 5 days, 1 month, and 3 months after surgery was 95.3%, 91.0%, and 90.7%, respectively, and age was a predictor of eGFR decline at 3 months after surgery. Our results suggest that off-clamp non-renorrhaphy open PN can be safely adopted in patients with impaired renal function. We have also performed off-clamp, non-renorrhaphy open PN for cT1b tumor (*37*). Appropriate hemostasis during and after tumor resection using SOFT COAG and hemostatic agents is mandatory to perform the surgery safely.

Soft coagulation for hemostasis

Soft coagulation or hemostatic agents are used for hemostasis after PN when cortical renorrhaphy is omitted. Although soft coagulation can lead to denaturation and necrosis of the renal parenchyma, shrinkage of the kidney volume after PN using soft coagulation is not well known. In the on-clamp setting, a favorable result in 1 postoperative month renal function is reported for the soft coagulation group compared with that in the double-layer renorrhaphy group (-3.5 mL/min *vs.* -13 mL/min, $p = 0.009$) (*39*). In this study, the shortened warm ischemic time (11.4 min *vs.* 20.3 min) might have also positively affected postoperative renal function. Intriguingly, an *in vivo* study in pigs revealed that renal parenchymal denaturation after soft coagulation reached a depth of 4 mm, and the temperature increased by 15.6 °C at a depth of 5 mm and 8.8 °C at 10 mm (*40*). Presumably, the effect of soft coagulation on ipsilateral renal function is not negligible. Further studies are necessary to clarify the effect of soft coagulation on renal volume.

In conclusion, off-clamp, non-renorrhaphy PN is safe for small renal tumors. Hilar control PN is reported to be feasible without an increased risk of severe complications; however, whether it deteriorates postoperative renal function remains controversial. Conversely, cortical renorrhaphy negatively affects renal function by damaging renal vessels and increasing the warm ischemic time. A prospective comparative study is required to verify these findings. Nevertheless, with the accumulation of clinical experience with off-clamp, non-renorrhaphy PN with a new hemostasis technology in robot-assisted settings, we may be one step closer to realizing the ideal PN.

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CORRESPONDENCE

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COVID-19 pandemic-altered epidemiology of respiratory syncytial virus and human metapneumovirus infections in young children

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Abstract: To evaluate the impact of the COVID-19 pandemic on the epidemiology of respiratory viral infections, we examined the prevalence of respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) infections for pediatric patients admitted to our hospital before and after the COVID-19 pandemic from January 2015 to June 2023. During the COVID-19 pandemic, no outbreaks of RSV infections were seen in 2020, and no outbreaks of hMPV infections were seen in 2020 and 2021. Before the pandemic, the two epidemics did not overlap, but after the pandemic, the two epidemics almost overlapped for the second year in a row. The average age of patients with both RSV and hMPV infection after the pandemic was significantly older than before the pandemic by approximately one year.

***Keywords*:** COVID-19, viral interference, respiratory syncytial virus, human metapneumovirus

Introduction

Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are closely related viruses belonging to pneumovirinae subfamily and cause bronchiolitis and pneumoniae in infants and young children, resulting in hospitalization, which becomes a major health problem in pediatric care (*1,2*). To evaluate the impact of the COVID-19 pandemic on the epidemiology of these viral infections, we investigated and compared the epidemic patterns of RSV and hMPV infections in children admitted and diagnosed at our hospital before and after COVID-19 pandemic from January 2015 to June 2023. The pathogenic diagnosis was made by antigen test before 2020 and by Filmarray® respiratory panel (ver2.1) test thereafter.

Our hospital is a tertiary emergency medical facility in the North Tama area of Tokyo, Japan. It has 611 beds, more than 20,000 annual admissions, more than 10,000 annual emergency transfers, and approximately 2,000 outpatients per day.

This study was approved by the Ethical Committee of Musashino Red Cross Hospital (approval number 4061). Informed consent was secured by opt-out method. It was performed in compliance with the ethical treatment policy of human and animal research participants and the latest Declaration of Helsinki.

Trends of RSV or hMPV infected patients under 10 years old who admitted in our hospital

In the reports so far, the epidemics of both virus infections have not completely overlapped in Japan (*3,4*), and the epidemic peaks of both infections in our hospital from 2015 to 2019 did not overlap as well as shown in Figure 1. These phenomena are referred to as social viral interference and have been mentioned in several viral infections (*5-7*). COVID-19, which emerged at the end of 2019, quickly spread around the world and became a pandemic (*8,9*). Japan also implemented a semi-social lockdown from April to May 2020. As a result, social activity restrictions continued, and no epidemics of RSV or hMPV were observed in 2020. After that, a large-scale epidemic of RSV was seen in the summer of 2021 due to the easing of restrictions on social activities and movements and the reopening of nursery schools, but no hMPV epidemic was observed. In 2022 and 2023, epidemics of both RSV and hMPV were seen.

Interestingly, both outbreaks occurred around the same time, unlike before the COVID-19 pandemic (Figure 1). More interestingly, when comparing the age distribution of infected children, the age distribution in post-COVID-19 epidemic shifted nearly one year older than that before COVID-19 epidemic (Figure 2). Prior to the COVID-19 pandemic, the age distribution during the

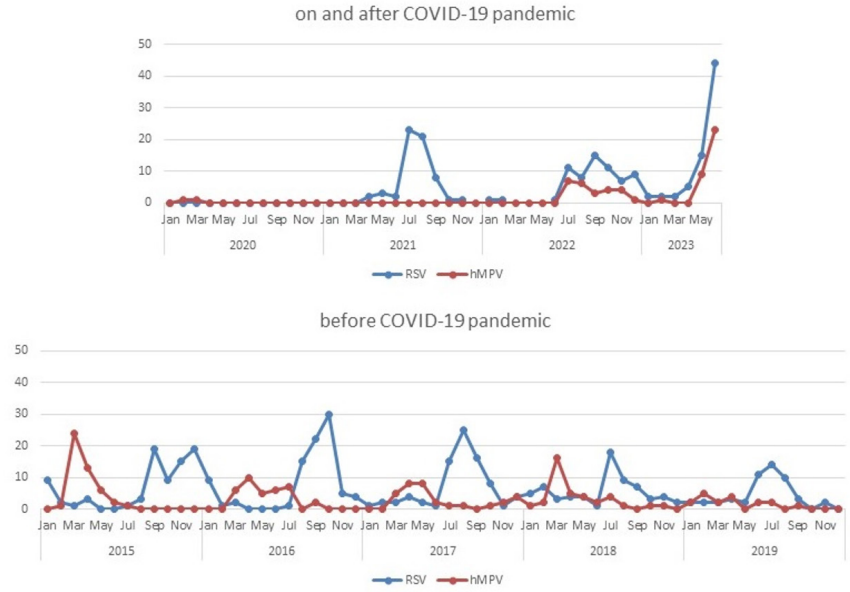

Figure 1. Trends of RSV or hMPV infected patients under 10 years old who admitted in our hospital were shown. Before COVID-19 pandemic, the epidemic peaks of RSV and hMPV infections did not overlap in each year. In 2022 and 2023, the epidemic peaks of both infections completely overlapped.

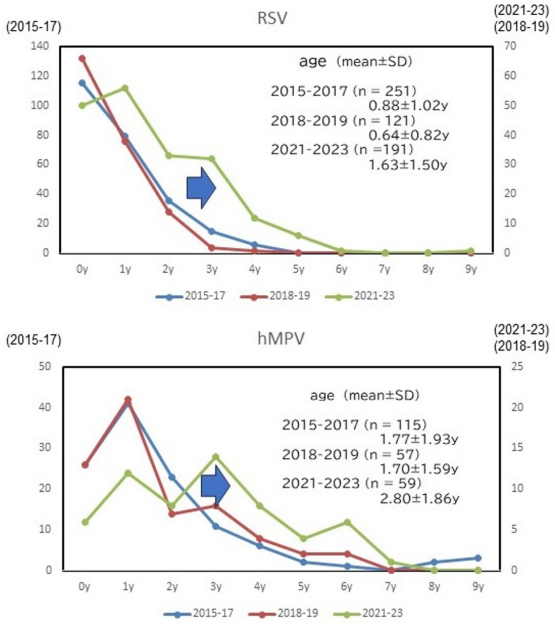

Figure 2. The age distribution of RSV (upper graph) and hMPV (lower graph) infected patients in three different periods (2015-2017, 2018-2019, and 2021-2023) were presented. The age distribution of 2021-2023 period is shifted to right (arrows) compared to that of 2015-2017 and 2018-2019 and the mean age of 2021-2023 is significantly older ($p < 0.001$: Mann-Whitney *U* test) than that of 2015-2017 and 2018-2019 in both RSV and hMPV infections.

epidemic of both viral infections was almost similar from year to year (data not shown).

COVID-19 pandemic-altered epidemiology of RSV or hMPV infections in young children

The periodic prevalence of epidemic respiratory viral infections in children can be attributed to several factors.

First is viral evolution. Viruses have the ability to mutate and evolve rapidly. These new strains can lead to recurrent outbreaks as the population lacks immunity to the modified virus. Second is host susceptibility. In a population, individuals may gain immunity to a specific viral infection through prior exposure or vaccination. However, over time, the immunity acquired through natural infection or vaccination can wane. Third are changes in population density and mobility. Population dynamics, including changes in population density and mobility, can influence the transmission of viral infections. Increased travel, urbanization, and global connectivity facilitate the rapid spread of viruses across regions and continents. Fourth are environmental factors. Certain viral infections exhibit seasonal patterns due to environmental factors. For instance, respiratory viruses like influenza tend to peak during the colder months when people spend more time indoors in close proximity, providing favorable conditions for viral transmission. Additionally, changes in climate patterns or ecological disturbances can affect the distribution and prevalence of vector-borne infections such as dengue or Zika virus. Fifth is lack of universal vaccination or treatment. The absence of effective vaccines or treatments against a particular viral infection can contribute to its periodic prevalence.

RSV and hMPV spread through similar routes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the measures taken to control COVID-19 have inevitably limited the transmission of both respiratory viruses among children. Furthermore, the reduced exposure to common respiratory viruses during the pandemic may affect the development of natural immunity in children, potentially leading to a susceptible population when restrictions ease and social interactions

increase. One of the reasons why children aged 3 to 4 years became more susceptible to RSV and hMPV after the COVID-19 pandemic may be that immunity was not stimulated due to the decrease in epidemic viral diseases during the COVID-19 pandemic. Also, the possibility that the virus mutated during the COVID-19 pandemic cannot be completely ruled out.

It is unlikely that the difference in virus detection sensitivity between antigen testing and PCR testing would have an impact on this observation. FA testing was performed at the time of admission for acute lower respiratory tract infection, and the clinical symptoms were all characteristic of RSV and hMPV infections. Furthermore, it is also unlikely that an increase in detection sensitivity would result in matching the timing of epidemics from the same reason. Between January 2021 and June 2023, there were only two cases in which both RSV and hMPV were positive by FA testing, and significant interference was observed between the two virus infections (odds ratio = 0.221, 95% CI: 0.053-0.916, $p < 0.05$ by Fisher's exact test).

Viral interference at the individual level has been verified in animal experiments (*10,11*). However, virus interference at the population level is observed as an indirect phenomenon and its causes are complicated. From this perspective, a detailed examination of the trends in infectious diseases, especially in children before and after the rare COVID-19 pandemic will provide very important suggestions for considering the mode of transmission of viral infections in society, the maturation process of immunity to viruses, and countermeasures against acute viral infection epidemics.

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LETTER

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Bridging the gap: International efforts and behavioral strategies to combat COVID-19 vaccine wastage

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Abstract: Highlighted by the G7 Hiroshima Summit and evident in Japan's complex vaccination program, the issue of novel coronavirus disease 2019 (COVID-19) vaccine allocation and utilization, particularly the dilemma of minimizing vaccine wastage, extends beyond national concerns. Various global strategies, such as using behavioral science principles like 'nudges', have been implemented to tackle the problem. However, scientific evaluation and international collaboration are insufficient; thus, analyzing successful case studies and innovative methods is crucial to pave the way for future preparedness and resilient responses to emerging pandemics.

***Keywords*:** Novel coronavirus disease 2019 (COVID-19), vaccine wastage, nudge, Japan, international collaboration

As discussed at the G7 Hiroshima Summit held in May 2023, the appropriate allocation and utilization of vaccines for novel coronavirus disease 2019 (COVID-19) remain international challenges (*1*). This issue was underscored when, as of March 2022, a surplus of 1.4 billion doses of vaccines was reported within the G7 countries, triggering a complex dilemma regarding the minimization of vaccine wastage. In response, several nations, including the United States, China, and Germany, embarked on concerted efforts to donate these surplus vaccines to developing countries (*2*). Japan also aligned itself with this mission, offering 1.24 million doses to Taiwan. However, a disconcerting minimum of 77.83 million doses of expired vaccines were reported to be discarded (*3*).

The situation in Japan adds a layer of complexity to this international concern. The vaccine program in the country was orchestrated by local governments, with the central government shouldering the financial responsibility for vaccine administration and waste disposal (*4*). This organizational structure led to local governments lacking sufficient incentives and accountability to curtail waste through monitoring cancellation rates or bolstering community vaccination rates. Further complicating matters, opposition from local governments resulted in vaccination rates being withheld from public view in 60% of prefectures on a municipality-specific basis. This lack of transparency may have contributed to a deceleration of the vaccination process, as competitive elements were absent, and the perception of scrutiny by others was limited.

It is evident that minimizing vaccine wastage transcends national concerns and emerges as an issue of paramount importance on the international stage. Potential strategies for addressing these issues could incorporate "nudges", which are behavioral science principles such as "loss aversion" and "social comparison" (*5*). Specifically, reassigning the financial burden of vaccine wastage to local governments and disclosing cancellation and wastage data to incite competition among municipalities. These 'nudges' have been validated in healthcare, demonstrating that cognitive bias-based strategies can be instrumental toward ideal public health policies (*5*).

Indeed, these strategies were used globally during the COVID-19 pandemic. In places like Oregon, United States, vaccines were administered in areas with high foot traffic, ensuring prompt accessibility for interested individuals (*6*). Waitlists and real-time cancellation disclosures were established in countries such as Germany, South Korea, and France, with France taking the lead through the "Covidliste" initiative (*7*). However, the scientific evaluation of these strategies is insufficient. With COVID-19 vaccine demand waning, the assessment of these efforts must become a priority in international collaboration (*8*).

In Japan, where the switch to effective pandemic countermeasures proved difficult amid social turmoil

over three years (*9*), the lessons from these strategies are particularly resonant. As people are often strongly inclined to maintain the status quo, especially during exhausting periods (*10*), understanding the benefits and limitations of nudge methods on a daily basis can help prepare for future pandemics.

In conclusion, the experiences from COVID-19 vaccine distribution necessitate a comprehensive review, moving beyond medical solutions to include an evaluation of successful case studies, innovative behavioral strategies, and international collaboration. This multifaceted approach to understanding vaccine wastage could pave the way for future preparedness and foster more resilient responses to emerging pandemics.

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LETTER

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Antibody levels and the risk of SARS-CoV-2 infection during the Omicron surge

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Abstract: We examined the association between antibody titer levels and risk of coronavirus disease 2019 (COVID-19) infection in the general Japanese population, including a total of 1,972 participants between June and September 2022. Specifically, we ascertained participants' IgG antibody titers targeting the spike protein and infection status, and subsequently examined the association between antibody titer categories (< 2,500, 2,500–5,000, 5,000–10,000 and > 10,000 AU/mL) and COVID-19 infection to estimate risk ratios (RR) and their 95% confidence intervals (CI). Compared to the lowest category, the adjusted RR for participants with antibody titers ≥ 10,000 AU/mL was 0.38 (95% CI: 0.20–0.71). The observed non-linear relationship between the titers and the risk of infection showed that the risk decreased as the participant's antibody titer increased, but the slope became milder when the antibody titer reached approximately 10,000 AU/mL. These findings may contribute to the use of an individual's antibody titer to consider appropriate timing of vaccination.

***Keywords*:** antibody titer category, COVID-19, general population, spike protein, vaccination

Vaccinations increase the levels of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Lower antibody titers were associated with the increased risk of infection during the Alpha and Delta surge (*1,2*), although few studies have examined this during the Omicron surge (*3,4*). A cohort study that included healthcare workers revealed that a lower antibody category divided by a specific value was associated with the increased risk during the Omicron surge (*4*). However, a more detailed assessment of the association between the levels of antibody titers and the risk is warranted in the general population, especially in the elderly.

The Bizen Coronavirus Disease 2019 (COVID-19) Antibody Test project (BCAT) is a community-based prospective cohort study that began on June 3, 2022, where antibody titers were measured every two months among residents of Bizen City, Okayama, Japan, or people working in the city. The study was approved by the Ethics Committee of Okayama University Hospital (No. 2205-061). We recruited a total of 1,972 participants over the age of 18. For this analysis, we included up to the second titer measurement from participants who had provided at least one measurement between June 3 and September 23, 2022. We excluded titer measurements if the vaccination was administered within two months of the measurement, yielding a total of 2,345 measurements from 1,649 participants. BA.5 subvariants of the Omicron variant were dominant during the period.

We collected fingertip whole blood samples (30 μL) and used the Mokobio SARS-CoV-2 immunoglobulin M (IgM) & immunoglobulin G (IgG) Quantum Dot immunoassay test kit (Mokobio Biotechnology R&D Center Inc., Rockville, MD, USA) to measure IgG antibody titers targeting the spike protein receptor-binding domain (*5,6*). We also confirmed whether the participants had tested positive for COVID-19 infection during the period between June 1 and September 25 and linked the infection status (*i.e.*, positive or negative) with the most recent antibody measurement information within two months. Since all positive cases that occurred in Okayama Prefecture were registered until September 25, we obtained the infection history from the Okayama Prefecture Registry and supplemented this with the self-reported questionnaire for those who did not live in the prefecture.

We examined the association between antibody titer category (< 2,500, 2,500–5,000, 5,000–10,000, and ≥ 10,000 AU/mL) and COVID-19 infection using a generalized estimating equation model accounting for a correlation within a participant, assuming a Poisson distribution with robust error variance. We then estimated risk ratios (RRs) and their 95% confidence intervals (CIs) adjusting for sex and age categories (18–39, 40–59, 60–79, and ≥ 80 years). We also evaluated the non-linear relationship between antibody titers

Table 1. A antibody titers and COVID-19 infection in Bizen city, Japan (n = 1,649)[a]

IgG (AU/mL)	COVID-19 infection case / Total measurement (%)	Adjusted RR (95% CI)[b]
< 2,500	105 / 1418 (7.4)	1.0 (ref)
2,500–5,000	14 / 340 (4.1)	0.57 (0.33–0.99)
5,000–10,000	8 / 266 (3.0)	0.42 (0.21–0.85)
≥ 10,000	9 / 328 (2.7)	0.36 (0.18– 0.71)

[a] We analyzed 2345 measurements from 1649 participants with no missing variables. [b] Adjusted for sex and age categories. CI, confidence interval; COVID-19, coronavirus disease 2019; IgG, Immunoglobulin G; arbitrary unit AU; RR, risk ratio.

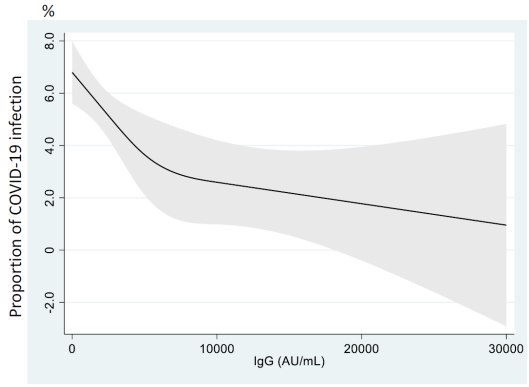

Figure 1. Association between immunoglobulin G (IgG) values (AU/mL) and the risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (%). The natural cubic spline that had three knots (2,500, 5,000, and 10,000 AU/mL) is shown as a black line and the 95% confidence intervals (CIs) are shown as a gray range. The upper limit of antibody titers was set at 30,000 AU/mL, as instructed by manufacturer's instructions; any value exceeding this limit was replaced into the upper limit. The shape of the spline curves did not change when we used different number of knots (*e.g.*, from 3 to 7).

(continuous) and the risk using natural cubic spline curves. All analyses were conducted with Stata SE version 17 (StataCorp LP, College Station, TX, USA).

A total of 136 of the 1,649 participants were infected during the study period. Higher antibody titers were associated with a lower risk. Compared with the lowest antibody category, the adjusted RR was 0.38 (95% CI: 0.20–0.71) for the participants with antibody titers ≥ 10,000 AU/mL (Table 1). The risk decreased as the antibody titer of the participant increased, although the slope became mild at antibody titers of approximately 10,000 AU/mL (Figure 1).

Despite several limitations, such as residual confounding due to underlying diseases or the assumption of the same antibody titer during the two months after the measurement, this study showed that higher antibody titers were associated with lower risk of infection in a dose-response manner during the Omicron surge. Moreover, the risk declined sharply at antibody titers of approximately 10,000 AU/mL. These findings could contribute to the use of an individual's antibody titer to consider appropriate timing of vaccination.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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