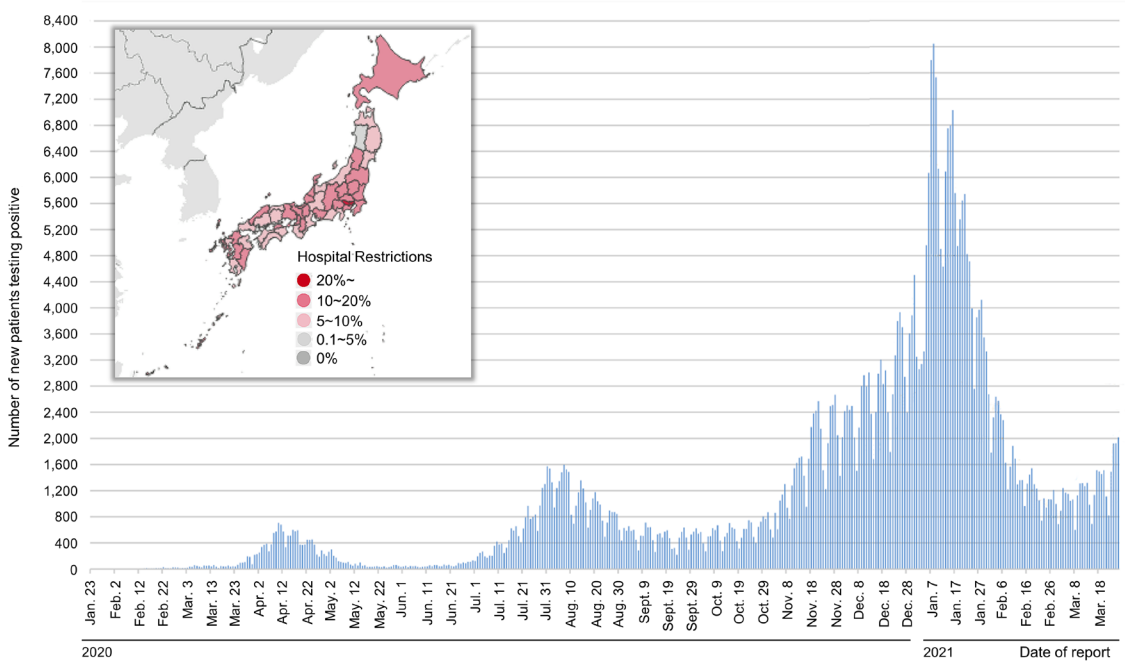
Print ISSN: 2434-9186 Online ISSN: 2434-9194

NCGM

Global Health & Medicine

Volume 3, Number 2
April, 2021

Special Topic: COVID-19



Daily number of newly confirmed COVID-19 cases and status of hospital restrictions in Japan. (Page 57)

Print ISSN: 2434-9186
Online ISSN: 2434-9194
Issues/Year: 6
Language: English

Global Health & Medicine

Global Health & Medicine

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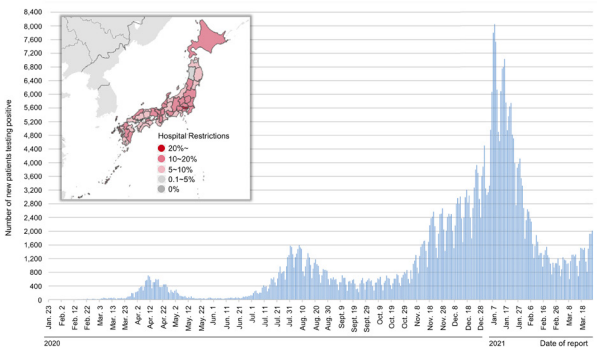

Daily number of newly confirmed COVID-19 cases and status of hospital restrictions in Japan. The rapid global spread of the COVID-19 pandemic has posed a significant challenge to various countries in terms of the capacity of hospitals to admit and care for patients during the crisis. In Japan, there have been three waves of substantial increases in the number of the infected so far. Although various measures are being actively implemented to slow the spread of the virus and reduce the strain on the health care system, the reality is that there are still a significant number of hospitals at risk of being overloaded in the event of a future surge in cases. (Data Source: *https://www.mhlw.go.jp/content/10906000/000760545.pdf*, *https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000164708_00001.html*) (Page 57)

Global Health & Medicine. 2021; 3(2):56-59.
EDITORIAL
DOI: 10.35772/ghm.2021.01031

Hospital capacity during the COVID-19 pandemic

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Abstract: The rapid global spread of the COVID-19 pandemic has posed a significant challenge to various countries in terms of the capacity of hospitals to admit and care for patients during the crisis. To estimate hospital capacity during the COVID-19 pandemic, clinicians working in tertiary hospitals around the world were surveyed regarding available COVID-19 hospital statistics. Data were obtained from 8 tertiary centers in 8 countries including the United States, United Kingdom, Switzerland, Turkey, Singapore, India, Pakistan, and Japan. The correlation between the number of patients with COVID-19 per 1 million population *vs*. the maximum number of inpatients with COVID-19 in a representative tertiary hospital in each country was determined, as was the correlation between COVID-19 deaths per 1 million population *vs*. the maximum number of patients with COVID-19 in the intensive care unit (ICU). What was noteworthy was that none of the 8 hospitals reduced emergency room (ER) activity even at the peak of the pandemic although treatment of patients without COVID-19 decreased by 0-70% depending on the extent of the epidemic. Although various measures are being actively implemented to slow the spread of the virus and reduce the strain on the health care system, the reality is that there are still a significant number of hospitals at risk of being overloaded in the event of a future surge in cases.

***Keywords*:** COVID-19, hospital, capacity, ICU, ER

Since the outbreak of COVID-19 in January 2020, the pandemic has struck Japan in 3 waves (Figure 1) (*1*). The third most recent wave was the largest, and hospital beds for patients with COVID-19 were almost fully occupied in large cities. In Tokyo, the medical system was literally on the verge of collapse. From a global point of view, however, there were relatively few patients with COVID-19 per population in Asia. According to recent global data, there were 3,587 patients with COVID-19 in Japan and 69 deaths per million population. Those numbers were much smaller than numbers in Western countries *e.g*. the United States (91,537 and 1,667, respectively) and the United Kingdom (62,895 and 1,849, respectively) (*2*).

The National Center for Global Health and Medicine (NCGM) is one of the 6 National Centers in Japan with a specified mission that includes dealing with infectious disease outbreaks. NCGM Center Hospital is a special function (tertiary) hospital with 700 beds including 4 beds in the high consequence infectious diseases (HCID) unit and 21 negative pressure beds. The NCGM Center Hospital has been expanding its capacity for COVID-19 patients depending on the patient load, with a peak capacity of 70 beds including 8 intensive care unit (ICU) beds (Figure 2). The Hospital had to allocate another 8 ICU beds for critical care patients without COVID-19 to continue functioning as a tertiary general hospital. There are more than 15 tertiary hospitals (most of which are University Hospitals) in the Tokyo metropolitan area, and they allocated a similar number of ICU beds for patients with COVID-19. In total, around 300 ICU beds were allocated, with additional ICU beds allocated in other city hospitals. According to the Tokyo Metropolitan Government, the number of patients with COVID-19 in the ICU (either on a respirator or ECMO) peaked at around 160 (*3*), and a collapse of the medical system was narrowly avoided.

As COVID-19 spreads rapidly around the country, a major concern has been the ability of hospitals to admit and care for patients (*4-8*). Many countries initially imposed stay-at-home orders and limited business activity in order to slow the spread of the virus and reduce the strain on health care systems, and these measures have been effective in managing the health crisis. However, there are still a significant number of hospitals at risk of being overloaded in the event of a future surge in cases.

In Western countries where patients with COVID-19 were much more numerous, a number of personal communications among clinicians have reported, say, that more than 200 patients were hospitalized for COVID-19 or that more than 50 patients were treated in the ICU of tertiary hospitals. Since the major of Japanese hospitals cannot sustain the treatment of so

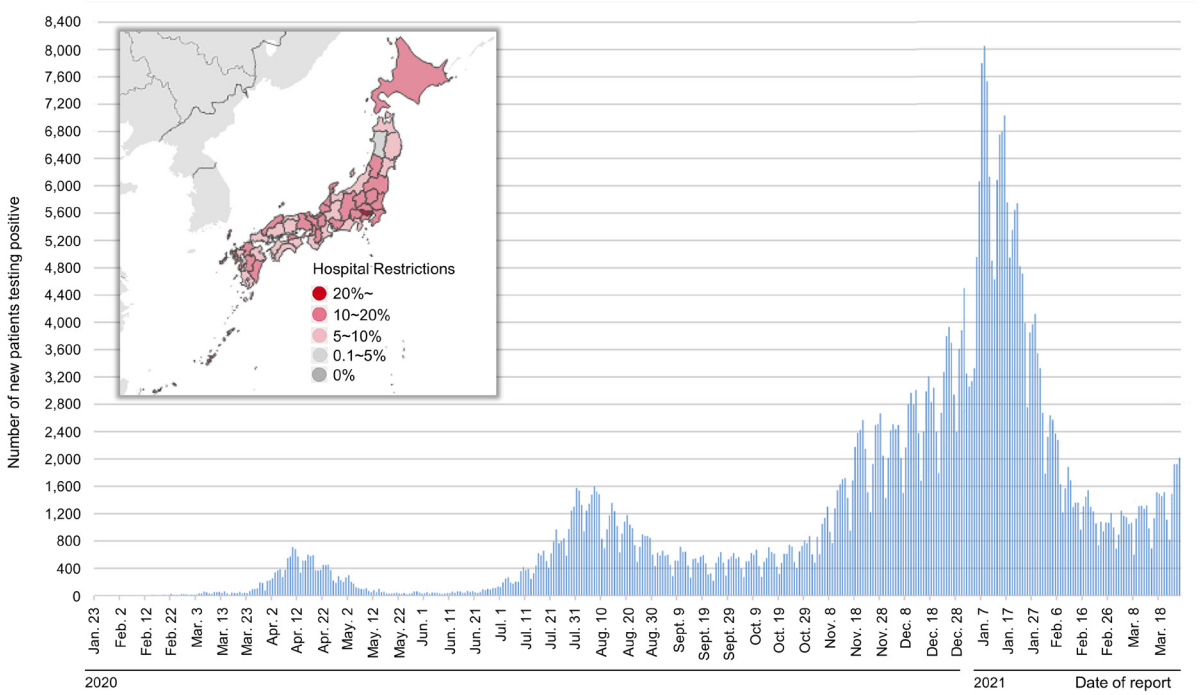

Figure 1. Daily number of newly confirmed COVID-19 cases and status of hospital restrictions in Japan. (Data Source: *https://www.mhlw.go.jp/content/10906000/000760545.pdf, https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000164708_00001.html*)

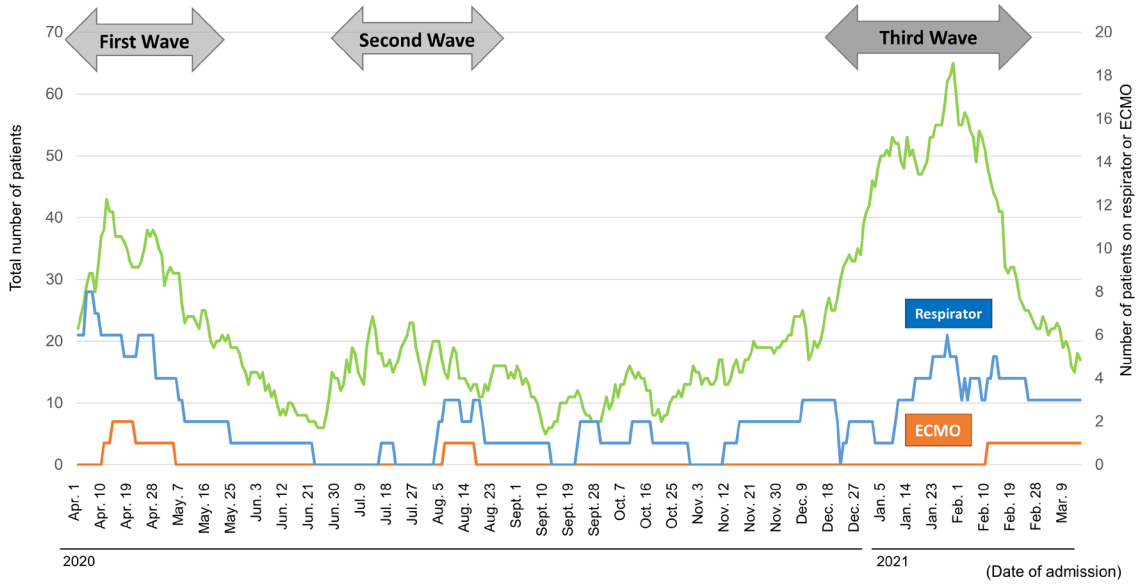

Figure 2. Number of patients with COVID-19 admitted to the NCGM Center Hospital (April 2020~March 2021). NCGM: National Center for Global Health and Medicine.

many patients with COVID-19, the current authors were very curious about the difference in numbers. However, obtaining official COVID-19 hospital statistics is difficult because such data are extremely sensitive and hospital administrators are generally very reluctant to disclose them. Therefore, clinicians working in tertiary hospitals around the world were personally contacted to inquire about available COVID-19 hospital statistics. Admittedly, the data obtained are not official hospital data and some may originate from internal personal communications. This is why all of these hospital data other than those from the NCGM have been kept anonymous except for nationality.

Data were obtained from 8 tertiary centers in 8

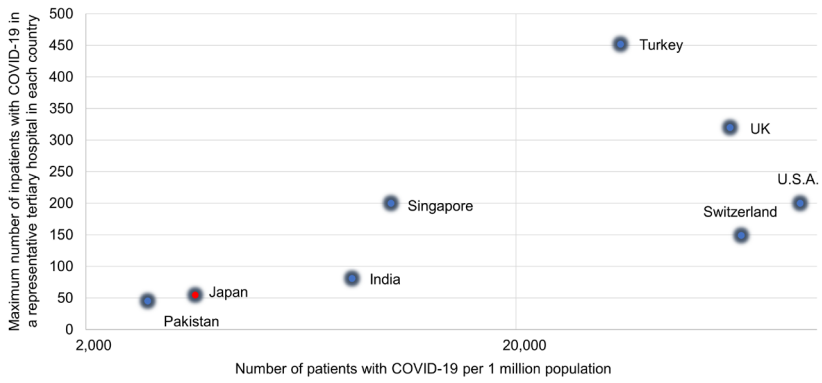

Figure 3. Number of patients with COVID-19 per 1 million population *vs.* maximum number of inpatients with COVID-19 in a representative tertiary hospital in each country.

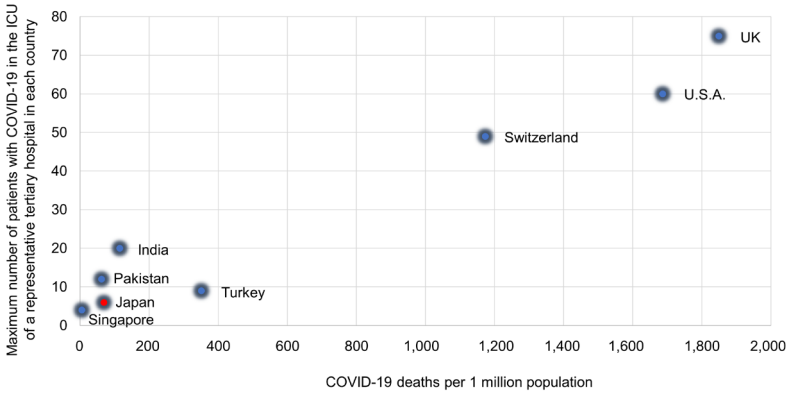

Figure 4. COVID-19 death per 1 million population *vs.* maximum number of patients with COVID-19 in the ICU of a representative tertiary hospital in each country.

countries. Figure 3 shows the correlation between the number of patients with COVID-19 per 1 million population *vs*. the maximum number of inpatients with COVID-19 in a representative tertiary hospital in each country. There was a roughly logarithmic correlation and Japan (the NCGM Center Hospital) is located in the bottom left of the chart. Figure 4 shows the correlation between COVID-19 deaths per 1 million population *vs*. the maximum number of patients with COVID-19 in the ICU. Asian centers including the NCGM Center Hospital were located in the bottom left of the chart and Western countries in the top right. Although the correlations in Figure 3 and Figure 4 are very rough ones, relatively few medical resources were deployed in Japan and the rest of Asia thanks to relatively small numbers of patients. However, expansion of hospital capacity in the event of a worsening epidemic may be difficult because of limited medical personnel in Japan. According to this survey, there were 3 to 5 times as many doctors in Western hospitals as there were in Japanese hospitals and 2-3 times as many nurses. Japan may urgently need the capacity to deal with a surge. What was noteworthy was none of the 8 hospitals reduced emergency room (ER) activity even at the peak of the pandemic although treatment of patients without COVID-19 decreased by 0-70% depending on the extent of the epidemic.

A point that need not be mentioned is that data from a single hospital cannot represent an entire country. Given the difficulty in obtaining such data, personal communications may provide useful information for formulating a strategy in the event of a pandemic. Although various measures are being actively implemented to slow the spread of the virus and reduce the strain on the health care system, the reality is that there are still a significant number of hospitals at risk of being overloaded in the event of a future surge in cases.

Acknowledgements

The authors would like to sincerely thank all of the clinicians who were involved in this study. Specifically, the authors would like to thank the following doctors for their assistance with the survey: Harshitha Mergey Devender, Ender Dulundu, Ghumza Farooq, Nermin Halkic, Masatoshi Hotta, Akiho Sugita, Hideaki Yano, and Kosuke Yasukawa (in alphabetical order).

Funding: None.

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

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Received March 28, 2021; Accepted April 8, 2021.

Released online in J-STAGE as advance publication April 11, 2021.

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Global Health & Medicine. 2021; 3(2):60-61.
EDITORIAL
DOI: 10.35772/ghm.2021.01035

SARS-CoV-2 surreptitiously injures the heart of Japanese: echocardiography is useful in evaluating cardiac damage

Issei Komuro*

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Abstract: Coronavirus disease 2019 (COVID-19) remains a threat worldwide over a year after the outbreak. Recently, several studies have reported that elevated serum troponin, which reflects myocardial injury, has a significant impact on worsening cardiovascular disease and the death of patients with COVID-19. In addition, magnetic resonance imaging (MRI) and echocardiography revealed abnormal myocardial findings in patients with COVID-19 who have recovered, as exemplified by a slight elevation of high-sensitivity troponin T (hsTnT). This editorial will discuss the impacts of SARS-CoV-2 on the heart of Japanese patients during infection and recovery and future perspectives.

***Keywords*:** COVID-19, SARS-CoV-2, myocardial injury, troponin, echocardiography

Coronavirus disease 2019 (COVID-19) has become a global pandemic. COVID-19 affects the cardiovascular system in various stages, increasing morbidity in patients with underlying cardiovascular conditions and causing myocardial damage and dysfunction. SARS-CoV-2 has been reported to enter the cell *via* angiotensin-converting enzyme 2 (ACE2), which is expressed in various cells of the heart including cardiomyocytes and endothelial cells (*1*). SARS-CoV-2 might injure cardiomyocytes directly and/or indirectly through endothelial cells. Patients with severe COVID-19 exhibit acute respiratory distress syndrome and a cytokine storm, increasing the risk of heart failure and thrombotic cardiovascular diseases, as well as elevated biomarkers such as cardiac troponin, NT-ProBNP, and D-dimer (*2-4*).

In a meta-analysis of 28 studies covering 4,189 patients with COVID-19, troponin levels were significantly higher in severely affected patients (*5*). Myocardial injury was more severe in hypertensive patients ($p = 0.03$) and the risk of mortality was also higher (risk ratio: 3.85-fold). During the course of the study, myocardial injury markers increased only in patients who died. Cardiac MRI scans revealed inflammatory findings in the myocardium in 4 (15%) of 26 athletes who had recovered from COVID-19 and prior myocardial injury in 8 more athletes (30%), leading to the recommendation that recovering athletes undergo cardiac MRI scans to return to competitive play (*6*). A multicenter study reported that half of the patients admitted with COVID-19 had some abnormal echocardiographic findings that affected their treatment options (*7*). Other studies have also reported that elevated troponin and comprehensive echocardiographic abnormalities such as entire LV dysfunction, wall motion abnormalities, diastolic dysfunction, RV dysfunction, and the presence of pericardial effusion affected all-cause mortality (*8*). Studies involving myocardial strain analysis using echocardiography have reported that abnormalities in left ventricular global longitudinal strain (LVGLS), right ventricular longitudinal strain (RVLS), and tricuspid annular plane systolic excursion (TAPSE) are independent predictors of in-hospital mortality in patients with COVID-19 (*9,10*).

In this issue, Dr. Hayama and colleagues reported on a study using echocardiography to analyze cardiac function in patients who recovered from COVID-19 (*11*). Of the 209 patients who recovered from COVID-19, 65% had elevated high-sensitivity troponin T (hsTnT), and LVGLS was reduced (< 20%) in 62 patients (29.7%), TAPSE was < 17 mm in 16 patients (7.7%), and right ventricular free-wall longitudinal strain (RVFWLS) was < 20% in 8 patients (3.8%). The decrease in LVGLS and RVFWLS was closely corelated with an increase in hsTnT. This finding clearly indicates that the heart is surreptitiously injured at a high rate in Japanese patients with COVID-19 and that echocardiography, including measurement of LVGLS in particular, is a useful method of detecting cardiac injury in patients who recovered from COVID-19.

SARS-CoV-2 could induce myocardial damage at a high rate even after a long period of recovery in Japanese as well as other ethnic groups, thus sounding an alarm for young Japanese who have few symptoms. Although whether myocardial damage will lead to

significant events remains to be elucidated, residual myocardial damage might cause arrhythmia such as ventricular tachycardia and atrial fibrillation as well as heart failure at a later date. Therefore, myocardial damage needs to be evaluated in patients with or without symptoms, and measurements of hsTnT and LVGLS with echocardiography are useful methods of evaluating that damage.

Funding: None.

Conflict of Interest: The author has no conflicts of interest to disclose.

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Received April 7, 2021; Accepted April 12, 2021.

Released online in J-STAGE as advance publication April 15, 2021.

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Global Health & Medicine. 2021; 3(2):62-66.

DOI: 10.35772/ghm.2020.01106

Investigator initiated clinical trial of remdesivir for the treatment of COVID-19 in Japan

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Abstract: Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originating in Wuhan, China, has spread globally very rapidly. The number of COVID-19 patients increased in Japan from late March to early April 2020. Since COVID-19 treatment methods with antiviral drugs were not established in March 2020, clinical trials began at a rapid pace worldwide. We participated in a global investigator-initiated clinical trial of the antiviral drug remdesivir. It took approximately two months to prepare for and start patient enrollment, 26 days to enroll all patients in Japan, and 32 days from the end of enrollment to the release of the first report, a fairly quick response overall. In the course of this clinical trial, we found some of the critical issues related to conducting an infectious disease clinical trial in Japan need to be addressed and tackled to support a rapid response. These included such things as the necessity of a research network to promote clinical research, a framework for a rapid review system of clinical trial notification, and better cooperation with outsourced teams. Furthermore, for Japan to take the lead in global collaborative research and development in the field of infectious diseases, it is necessary to develop further human resources and organization on a national basis. It is indispensable for Japan to establish a clinical trial system at the national level to prepare for future emerging and re-emerging infectious diseases.

***Keywords*:** SARS-CoV-2, clinical trial, infectious diseases

Introduction

The novel coronavirus identified in Wuhan, China, in December 2019, was named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It spread throughout the world as a new type of coronavirus disease (COVID-19) and became a major threat to public health and the economy (*1*). In Japan, the first case of a positive SARS-CoV-2 polymerase chain reaction (PCR) test was reported by the Japanese Ministry of Health, Labor and Welfare (MHLW) on January 16, 2020 (*2*), and the number of cases subsequently increased from late March through early April 2020. The first wave of cases peaked in early April, with more than 600 notifications of infection per day, and the number rapidly declined to around 20 per day in late May (*3*). The second wave has passed the peak in August, and Japan again faces the resurgence of COVID-19, which brought a record number of daily cases, 4,322, as of December 31, 2020.

Because COVID-19 is an emerging infectious disease and treatment methods with antiviral drugs were not yet established in March 2020, clinical trials had begun at a rapid pace around the world, and many are still ongoing. Remdesivir was found to have anti-SARS-CoV activity in a mouse infection model (*4*) and was offered for compassionate use in COVID-19 patients worldwide (*5,6*).

We participated in the U.S. National Institutes of Health (NIH)-led investigator-initiated clinical trial, "A Multicenter, Adaptive, Randomized Blinded Controlled Trial of the Safety and Efficacy of Investigational Therapeutics for the Treatment of COVID-19 in Hospitalized Adults", as a site and enrolled 15 cases. This trial showed that the use of remdesivir in COVID-19 patients accelerated time to recovery compared to that of a placebo (*7,8*). As a result, the U.S. Food and Drug Administration (FDA) issued an emergency use authorization for remdesivir on May 1 (*9*), and the MHLW has granted fast-track approval for remdesivir as the first treatment for COVID-19 on May 7, 2020 (*10,11*). The FDA approved a new drug application for remdesivir to treat COVID-19 requiring hospitalization on October

22, which allowed remdesivir for sale and marketing in the U.S (*12,13*).

This investigator-initiated clinical trial process was conducted very quickly, at a time when the number of patients infected with SARS-CoV-2 was high, allowing for a large number of case enrollments. To promote clinical trials in the field of infectious diseases in Japan, we reviewed the domestic process from study participation to reporting results, and we identified issues and sought improvements for the rapid implementation of future trials.

Process of the investigator-initiated clinical trial

In early February 2020, the U.S. NIH consulted with the Japanese MHLW about participating in an investigator-initiated international clinical trial of antiviral therapy for COVID-19, and our center, the National Center for Global Health and Medicine (NCGM), agreed to participate in the study. The NIH submitted the first protocol to the FDA on February 18, and the FDA issued a notification that it was safe to proceed on February 19. We participated in this study in the framework of an investigator-initiated clinical trial based on the Japan-Good Clinical Practice (J-GCP) and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use-GCP (ICH-GCP).

With detailed discussions with the MHLW and the Pharmaceuticals and Medical Devices Agency (PMDA), we submitted our initial clinical trial protocol to the PMDA on February 28. At the same time, we built a research team with the NCGM members and external contractors, and we welcomed a five-member NIH research support team to the NCGM from March 9-19. During the support team's visit to Japan, we worked together to prepare for the clinical trial in accordance with ICH-GCP, including contract signing, clinical trial procedures, in-house microbiology, clinical laboratory systems, investigational drug management procedures, online patient enrollment, staff training, and completion of various documents. We also translated the clinical trial protocol and manual of procedures into Japanese and prepared documents such as an informed consent form in line with J-GCP. After a site initiation visit by a monitoring team sent by the NIH on March 24, our center was approved by the NIH as a clinical trial site on March 25. After the delivery of the investigational drug on March 25, the first patient enrollment at our center was conducted on March 26. With the cooperation of each department, we completed the enrollment of 15 patients at our center on April 20 at 0:00 (Eastern Standard Time). After exchanging data cleansing queries with the NIH team, the “Remdesivir for the Treatment of Covid-19-Preliminary Report" was published in *The New England Journal of Medicine* on May 22, 2020 (*7*).

Why the clinical trial was conducted so quickly

The preparation of an investigator-initiated clinical trial generally takes a large amount of time when considering the process from concept development to research proposal creation, research budget securing, coordination with pharmaceutical companies, research system building, clinical trial consultation, contracting, *etc*. There are several reasons why we were able to achieve this clinical trial at an unprecedented speed. First, and most importantly, the MHLW, PMDA, NCGM staff, Institutional Review Board (IRB) members, and external contractors worked as a team under the direction of the Japanese government to prepare for this clinical trial and enroll patients.

The second reason was the support from the multidisciplinary specialist team from the NIH that stayed in Japan for 11 days to support the preparation of this clinical trial. Of the five members of the NIH team, two were coordinators, one was a microbiologist, one was a pharmacist, and one was a Japanese researcher working on infectious diseases who also served as an interpreter. The coordinator, microbiology technician, and pharmacist answered technical questions from our team while communicating within the NIH team. The Japanese researcher who served as a bridge between the NIH support team and the NCGM team played an important role in the progress of the trial, because it was sometimes difficult for our team to communicate in English for detailed information. Even after the NIH support team returned to the U.S., our team obtained technical advice *via* weekly online meetings; this contributed to the smooth operation of the process. The role of the NIH support team in the rapid preparation and smooth operation of the clinical trial was significant.

Lastly, prompt and flexible review of clinical trial notification by the PMDA was critical to the flow and speed of the preparation process (Figure 1). The PMDA expedited the investigation by swiftly responding to consultations from our team utilizing phone and email and allowing us to submit required documents flexibly, considering the challenging situation in which the study stood. Moreover, we were able to start patient enrollment in one month after the protocol submission for the following reasons: First, the MHLW allowed the clinical trial to begin without waiting for the 30 day investigation period setup for the investigation by the PMDA to be completed (*14*) and second, the PMDA spent a great deal of effort in conducting the investigation in a timely manner. Rapidly starting a clinical trial would have been impossible without the shortened 30-day investigation by the MHLW and the prompt review by PMDA.

Challenges for future clinical trials

The number of cases of SARS-CoV-2 in Japan increased rapidly from late March to early April 2020 and then declined rapidly. If rapid trial preparation and patient enrollment did not take place, the opportunity to find a

treatment method might have been compromised. In the course of this clinical trial, we incurred some challenges in Japan, but we also looked for ways to solve them (Table 1).

First, it is desirable to establish a research network in the field of infectious diseases to support implementing clinical trials, especially administrative work. This type of network has already existed for other diseases in Japan, *e.g.*, the Japan Clinical Oncology Group (JCOG) for cancer treatment research. Due to the shortage of human resources throughout the COVID-19 outbreaks, investigators needed to prepare for this clinical trial while dealing with patients in the medical field. We have realized that administrative work, which is a key to conducting clinical trials without interruption, requires heavy workloads. Fortunately, the team could ask for support from the Center for Clinical Sciences in the NCGM to handle the work. Nevertheless, considering the future outbreaks of infectious diseases, we urgently need to establish a research network to lead administrative tasks for conducting clinical research smoothly.

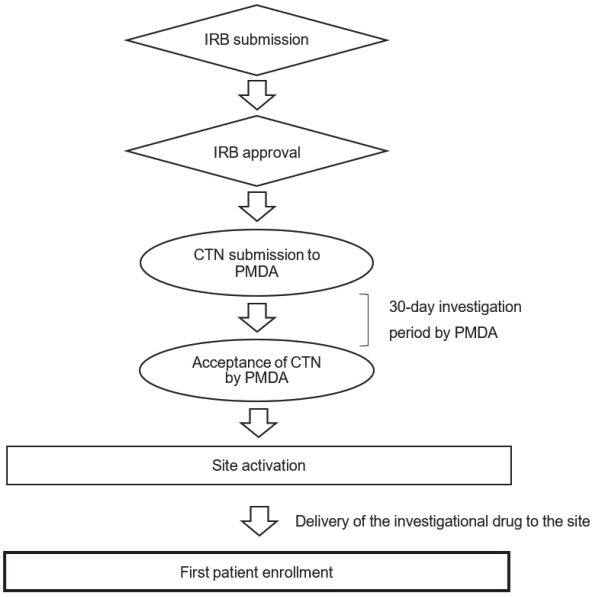

Figure 1. Approval process of investigator-initiated clinical trials in Japan. IRB: Institutional Review Board; CTN: Clinical Trial Notification; PMDA: Pharmaceuticals and Medical Devices Agency.

Second, the investigation period for clinical trial notification in Japan needs to be shortened for responding to an emergency. In this clinical trial, as the PMDA regarded the trial as a special case and made efforts to enable the investigation to be conducted exceptionally quickly, the trial launched without significant delay. Since the SARS-CoV-2 outbreak has proven that infectious diseases can sometimes lead to a rapid increase in the number of patients and have a tremendous social impact, it is necessary to have a first-track review system for clinical trial notification in place to ensure that clinical trials are carried out promptly.

The third issue concerns Site Management Organizations (SMOs), to which we often outsource some duties when conducting investigator-initiated clinical trials. In this clinical trial, it was challenging to reach a contract agreement with SMOs, which could not access sufficient safety information for COVID-19 in the early phase of the pandemic and estimated the risk of infection of the dispatched clinical research coordinators (CRCs) as very high. Although we, fortunately, found a company willing to take the job for the trial, it took more time and cost than usual for commissioning. Discussions need to be continued on how to convince SMOs to commit to clinical trials for infectious diseases after understanding the risk.

The fourth issue is the retention of signed informed consent forms. Because patients must sign the consent document before participating in a clinical trial following

Table 1. Challenges in clinical trials for emerging or re-emerging infectious diseases in Japan

No.	Challenges	Mitigation plans
(1)	Absence of a system to lead administrative tasks for clinical research programs in the field of infectious diseases.	It is desirable to establish a research network leading clinical research and supporting administrative tasks to accelerate clinical research for diagnostic technologies and treatment methods development.
(2)	In the case of outbreaks, the investigation period for clinical trial notification must be shortened.	Consider a fast-track review system as an alternative process.
(3)	Because of insufficient information of a new pathogen, especially in the early phase of the pandemic, it is difficult to recruit SMOs that support clinical trials.	Provide the latest accurate information for SMOs. It still needs to discuss how to convince SMOs to commit to clinical trials for infectious diseases after understanding the risk.
(4)	Retention of the contaminated informed consent forms after signing.	Put the method of using an electric document, which is now allowed by the MHLW, into practice. There are still challenges in using the new method in the medical field. e.g., installing an information network system to store and transfer confidential data securely.
(5)	Lack of capacities and experiences to conduct international clinical trials. The shortage of study specialists and the absence of a coordinate system for the trial is critical.	To strengthen the capacity to carry out and lead international clinical trials, Japan must develop a national coordinate system and promote more human resources development.

Japanese law, it became a problem for the investigators to store signed papers potentially contaminated by droplets from patients. Since the reduction of infectivity after 72 hours was reported (*15*), we isolated the paper documents for at least 72 hours before preserving them. On April 7, 2020, the MHLW announced that, under certain conditions, consent forms signed by patients could be reiterated in the form of electromagnetic records of documents, and electronically signed consent documents (*16*). If we had been able to use this method, the trial would have run more smoothly. Although the rule has been ready, using the method is limited to medical facilities and research institutes capable of setting up and managing an information network system to handle highly confidential personal data. We need to continue the ongoing discussion on putting this method in practice widely.

Finally, if Japan takes the lead in international clinical trials, it will be indispensable to develop a coordinated system at the national level and train more study specialists. The U.S. NIH dispatched expert teams to other countries early in the epidemic. In this trial, we accepted a team with specialists, including a microbiologist, a pharmacist, and a liaison who supports teams bridging the gap between a site and the NIH. Also, outsourced teams were utilized to organize human resources. Significant research funding allows the U.S. NIH to coordinate many global clinical trials hiring people with a high level of expertise. It is considered essential to develop experts who lead in the field of clinical research and build a national organization to coordinate international collaborative clinical studies so that Japan plays a leading role in international collaborative clinical research.

Conclusion

We participated in a clinical trial of remdesivir for the treatment of COVID-19 in Japan through a concerted domestic collaboration and support from the NIH. It took approximately two months to prepare for and start patient enrollment, 26 days to enroll patients, and all of this was done quickly, with 32 days from the end of registration to the release of the first report. Considering that the first wave of COVID-19 patients peaked in early April, and the number of cases declined rapidly after that, speed is critical when we conduct clinical trials for infectious diseases. To address second and third emerging and re-emerging infectious diseases, Japan needs to establish a clinical trial system of infectious diseases. The system must include organizations and a research network to promote research, a framework for the rapid review of clinical trial notification, and cooperation with SMOs. Moreover, for Japan to take the lead in global collaborative research and development, it is necessary to develop human resources and organization on a national basis to coordinate clinical trials over countries.

Acknowledgements

We acknowledge Ms. Miwa Izutsu and all team members who were involved in this clinical trial.

Funding: This work was supported by Health and Labor Sciences Research Grant, “Emerging and Re-emerging Infectious Diseases and Immunization Policy Promotion” (grant no. 19HA1003).

Conflict of Interest: S.S. received a grant from Shionogi, and N.O. received grants from Sanofi Pasteur. Other authors have no conflicts of interest to disclose.

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Received November 25, 2020; Revised January 27, 2021; Accepted February 4, 2021.

Released online in J-STAGE as advance publication February 11, 2021.

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Global Health & Medicine. 2021; 3(2):67-72.
MINI-REVIEW
DOI: 10.35772/ghm.2020.01117

COVID-19 and bronchial asthma: current perspectives

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Abstract: Angiotensin converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2), two receptors on the cell membrane of bronchial epithelial cells, are indispensable for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. ACE2 receptor is increased among aged, males, and smokers. As smoking upsurges ACE2 expression, chronic obstructive pulmonary disease (COPD) patients are prone to SARS-CoV-2 infection, and are at a higher risk for severe forms of COVID-19 (coronavirus disease 2019) once infected. The expression of ACE2 and TMPRSS2 in asthma patients is identical (or less common) to that of healthy participants. ACE2 especially, tends to be low in patients with strong atopic factors and in those with poor asthma control. Therefore, it could be speculated that asthma patients are not susceptible to COVID-19. Epidemiologically, asthma patients are less likely to suffer from COVID-19, and the number of hospitalized patients due to exacerbation of asthma in Japan is also clearly reduced during the COVID-19 pandemic; therefore, they are not aggravating factors for COVID-19. Related academic societies in Japan and abroad still lack clear evidence regarding asthma treatment during the COVID-19 pandemic, and recommend that regular treatment including biologics for severe patients be continued.

***Keywords*:** coronavirus disease 2019 (COVID-19), ACE2, ICS, TMPRSS2, biologics

Introduction

In corona viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the genome RNA is surrounded by an envelope consisting of a lipid bilayer and an outer membrane protein. SARS-CoV-2 initiates invasion into human cells after the Spike protein (S protein, in the envelope) binds to the cell membrane receptor, angiotensin converting enzyme 2 (ACE2). The S protein is cleaved into S1 and S2 by a human cell-derived protease. Then S1 binds to the ACE2 receptor. The other fragment (S2) is cleaved by transmembrane protease serine 2 (TMPRSS2), a serine protease on the surface of human cells, and as a result, membrane fusion proceeds (*1*). The gene expression of ACE2 and TMPRSS2 differs depending on the type of tissue/cell. ACE2 is widely expressed in the epithelial cells of the lungs, heart, small intestine, kidney, testicle and liver. TMPRSS2 is also widely expressed in the lungs, kidney, testicle and small intestine. Expression of ACE2 and TMPRSS2 in bronchial epithelial cells is essential for SARS-CoV-2 infection (*2*).

The expression level of ACE2 is influenced by age, sex, and lifestyle. ACE2 on the cell surface increases with age and generally tends to be denser in males than in females. In addition, the expression level of ACE2 is said to increase with exercise and smoking. The kidney has a high expression level of ACE2 and TMPRSS2. Although there are many reports of acute renal failure during COVID-19 pandemic, there is currently no clear evidence as to whether SARS-CoV-2 directly infects organs outside the respiratory system (such as the kidneys) (*3*). The expression level of ACE2 correlates with the susceptibility of infection by SARS-CoV-2, and is associated with increased morbidity in patients with respiratory diseases related to smoking. In COVID-19, complications such as hypertension, diabetes mellitus, cardiovascular diseases, chronic renal failure, malignancies (especially in those receiving chemotherapy and radiation therapy) and chronic respiratory diseases are at risk of aggravation.

ACE2 receptor and respiratory diseases

Since smoking significantly increases the expression of ACE2, patients with chronic obstructive pulmonary disease (COPD, caused by smoking in 90% of the cases in Japan) are also reported to have increased expression of ACE2 in the airway epithelium (from the bronchial region to the alveoli) (*4*). This is similar in both current and past smokers. Previous reports show that in COPD patients, airflow limitation progresses and the severity increases with the ACE2 expression level. Epidemiologically, COPD patients are known to be susceptible to COVID-19 and once contracted, the infection is known to be more severe, and correlates

with ACE2 expression levels (*5*).

Among chronic respiratory diseases, it has been reported that ACE2 expression is increased in idiopathic pulmonary fibrosis (IPF) as well as COPD (*6*). Among patients with interstitial pulmonary pneumonitis, reports revealed a significantly higher risk of death in IPF patients (especially those with obesity) (*7*), and is also presumed to be closely related to ACE2 expression. On the contrary, it has been shown that ACE2 expression tends to be low in sarcoidosis (*6*). In the following paragraph, we focus on the findings of the expression of each receptor in bronchial asthma.

Bronchial asthma and ACE2 andTMPRSS2 receptors

According to many epidemiological studies in Japan, the smoking rate in asthma patients is 20%-40%, which is the same in healthy individuals. It is presumed that smoking is not involved in the expression of these receptors in asthma patients.

Peters *et al.* investigated the expression of each receptor using induced sputum in 330 asthma patients (about 60% of whom were severely ill) who participated in Severe Asthma Research Program-3 (SARP-3). The expression of ICAM-1 (intercellular adhesion molecule-1; the target receptor for rhinovirus and most frequent cause of virus-infection induced exacerbation in asthmatics) was significantly increased in asthma patients compared to 79 healthy individuals, whereas no difference is reported in the expression of ACE2 and TMPRSS2 between the asthma patient group and healthy individuals (*8*). Sub-analysis has shown that ACE2 expression among asthma patients is significantly increased in the elderly, males, and Africans. Furthermore, stratifying the asthma patients based on the amount of ICS (inhaled corticosteroids) used, it was shown that ACE2 and TMPRSS2 expression was significantly lower in high-dose users than in non-users and low/medium-dose users. On the other hands, Jackson *et al.* examined the nasal epithelium of 318 pediatric asthma patients and reported that ACE2 expression was significantly lower in patients with high IgE levels and strong atopic factors (*9*). At the same time, it has been shown that ACE2 expression is significantly reduced in both the nasal and airway epithelium after the post-bronchial allergen-challenge *in vivo* and incubation with interleukin-13 (IL-13) *in vitro*. In addition, Kimura *et al.* examined the nasal mucosa of rhinitis patients and reported that ACE2 expression was significantly lower in patients with stronger Th2 inflammation, and poorer control of comorbid asthma (*10*).

In summary, the expression of ACE2 and TMPRSS2 is not higher in asthma patients than in healthy subjects. Moreover, ACE2 tends to be low in patients with poor asthma control, strong atopic factors, and high-dose ICS use. From these facts, it could be speculated that asthma patients are not particularly susceptible to COVID-19 and do not become severely ill.

COVID-19 and asthma: findings from epidemiological studies

Bronchial asthma is a chronic respiratory condition of variable severity characterized by reversible airflow obstruction, airway hyper-responsiveness, and inflammation which results in symptoms such as wheezing, breathlessness, and coughing. In Japan, approximately 3 million people are affected by asthma, and among these, 30% have moderate asthma, while 7% have severe asthma. The prevalence of asthma has increased, while its mortality has decreased (1.3 per 100,000 patients in 2018) after introduction of anti-inflammatory maintenance treatment mainly by ICS (*11*). The aim of asthma management is symptom control, and prevention of future risks. Thus, it is important to alleviate airway inflammation and prevent exacerbations which may induce airway remodeling and lead to an intractable condition.

In asthma patients, the antiviral immune response is inadequate, *i.e.* decrease in the capacity of IFN-α production during a viral infection, compared to healthy individuals; thus, the symptoms are generally exacerbated by respiratory virus infection. Rhinovirus and respiratory syncytial virus are the most common viruses that cause exacerbations, but it is also known that human coronaviruses (four types) are among the most common causes of exacerbation, accounting for about 10% in all (*12*). Based on this, theoretically, asthma patients are likely to be infected with SARS-CoV-2 and become more severely ill. However, there are few reports of exacerbation of asthma for the same family of coronaviruses such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (*13*).

From an integrated analysis of eight observational studies conducted in China, the United States and Mexico, Matsumoto *et al.* found that patients with COVID-19 had an asthma complication rate of 5.3%, lower than the average asthma prevalence of 8.0% in each region and asthma patients were shown to be less susceptible to COVID-19 (*14-16*). Other cohorts from the Western nations have also reported less asthma complications in COVID-19 patients requiring hospitalization (*17,18*). Beurnier *et al.* reported that 37 (4.8%) of the 768 hospitalized patients had asthma complications, but none experienced exacerbations of asthma itself during hospitalization (*19*). Among the asthma patients, 84% had a Body Mass Index (BMI) of 25 or higher, and a 59% had other comorbidities such as hypertension (27%) and diabetes (19%). Chhiba *et al.* used a computer algorithm in the US to search medical records of patients with COVID-19 confirmed by Polymerase Chain Reaction (PCR) and

Table 1. Numbers and percentages of asthma and COPD comorbidity in patients with COVID-19

Region	No. of COVID-19 patients*	Mean or median age (y)	No. of comorbid patients (%)		Regional asthma prevalence**	*Ref.*
			Asthma	COPD		
Wuhan, China	140	57	0 (0)	2 (1.4)	6.4 %	Wu F, *et al.* (*37*)
Wuhan, China	548	60	5 (0.4)	17 (3.1)		Li X, *et al.* (*14*)
Wuhan, China	1,590	49	0 (0)	24 (1.5)		Guan WJ, *et al.* (*38*)
Georgia, USA	305	60	32 (10.5)	16 (5.2)	7.7 %	Gold JAW, *et al.* (*39*)
California, USA	54	54	3 (0.6)	0 (0)		Hartmann S, *et al.* (*40*)
New York, USA	5,700	63	513 (9)	308 (5.4)		Goyal P, *et al.* (*21*)
New York, USA	1,651	50	99 (6)	66 (4)		Singer AJ, *et al.* (*15*)
Chicago, USA	1,526	59	220 (14.4)	NA		Chhiba KD, *et al.* (*20*)
Strasburg, France	106	64	23 (21.6)	NA	10.6 %	Grandbastien M, *et al.* (*17*)
Paris, France	768	54	37 (4.8)	NA		Beurnier A, *et al.* (*19*)
Korea	7,272	56	686 (9.4)	NA	3.9 %	Lee SC, *et al.* (*22*)
Italy	2,000	61	42 (2.1)	NA	6.5 %	Caminati M, *et al.* (*23*)
Total	21,660	-	1,660 (7.7)	433 (2)	7.5 %	-

*The numbers of patients were calculated only if the total numbers of patients and percentages were presented. **Regional asthma prevalence data are cited from The Lancet. 2019; 394:407-418; and CDC, 2020, most recent national asthma data, *http://www.cdc.gov/asthma/most_recent_national_ asthma_data.htm*.

performed a meta-analysis on the association between asthma/ICS use and hospitalization risk (*20*). Among the 1,526 patients with COVID-19, 220 (14.4%) had asthma, but were not associated with an increased risk of hospitalization in a model adjusted for age, gender, and comorbidities. At the same time, the results showed that the use of ICS did not increase the risk of hospitalization. From the above, it could be concluded that asthma patients are less likely to get infected with SARS-CoV-2, and even if they do, asthma exacerbation is unlikely to occur (summarized in Table 1).

A retrospective cohort study of patients admitted to two New York centers found no significant difference in asthma complication rates between non-severe COVID-19 patients (12.2%) and severe cases needing mechanical ventilation (13.1%) (*21*). An integrated analysis by Matsumoto *et al.* reported that the complication rate of COPD and diabetes was higher in severe cases than in non-severe cases of COVID-19, but there was no difference in the complication rate of asthma (*16*). On the contrary, according to the Korean nationwide retrospective cohort study using a health insurance database, concomitant asthma was not a significant risk factor for respiratory failure or mortality among all COVID-19 patients (odds ratio [OR]: 0.99, $p = 0.997$ and OR: 1.06, $p = 0.759$) after adjusting for age, sex. However, a history of acute exacerbation in the previous year before COVID-19 was a significant risk factor for death among COVID-19 patients with asthma (OR: 2.63, $p = 0.043$), especially for the elderly and males (*22*). Although few patients suffer from COVID-19, many who become severely ill reported that they were originally severely ill according to Global Initiative for Asthma (GINA) treatment step 4/5 (*23*). From these reports, asthma is generally not a severity factor for COVID-19, but due to the limited evidence in critically ill patients, the US CDC (Center for Disease Control) has stated that "Having moderate-to-severe asthma might increase risk for severe illness from COVID-19" (*24*).

There are limited reports on the current situation in Japan. At our hospital (National Center for Global Health and Medicine), 11 (5.5%) in-patients from March to August 2020 had a history of asthma. Three patients became ill enough to require oxygen administration, but showed immediate improvement, and there were none that showed exacerbation of asthma. Abe *et al.* compared and examined changes in the number of patients admitted to acute care hospitals in Japan due to asthma from 2017 to 2020 (*25*). In 2020, the number of hospitalizations until mid-February (when the first COVID-19 deaths were reported) remained unchanged compared to the previous three years, but since then until the end of May (when the government lifted the state of emergency all over Japan) the number of hospitalizations showed a significant 66% decrease (95% confidence interval [CI], 0.37-0.55; $p < 0.001$). The number of asthma hospitalizations during the pandemic continued to decrease, probably not due to the virus characteristics described above but to the increased preventive measures over this period. Individual-level hygiene measures to prevent COVID-19 might reduce exposure to the strong drivers of asthma exacerbations *i.e.* infection, allergens exposure, and air pollutants. Moreover, preventive behaviors such as quitting smoking, and better adherence to preventive medications (*26*) are important possible mechanisms for this drop in asthma hospitalizations.

Treatment for asthma during the COVID-19 pandemic: recommendations from guidelines

It was initially pointed out that ICS (the basis of asthma treatment) has two potential risks: increasing morbidity,

and aggravating pneumonia and ARDS after infection. On the contrary, when stratifying patients according to ICS usage, it is suggested that there is a possibility of preventive effect as ACE2 and TMPRSS2 are significantly decreased in high-dose users compared with non-users and low/medium dose users (*8*).

A systematic review by Halpin *et al.* stated that there is currently no evidence supporting the fact the use of ICS is detrimental or beneficial to the outcome of COVID-19 (*27*). In an observational study by Schultze *et al.*, the risk of death was examined in the asthma cohort (n = 818,490) with preparations containing ICS *vs.* short acting β2-agonists, and in the COPD cohort (n = 148,557) with preparations containing ICS *vs.* long acting muscarine agonists/long acting muscarine β2-agonists (*28*). The COPD cohort had a significantly increased risk of COVID-19-related death in the ICS group, while the asthma cohort had a higher risk of death with high-dose ICS, but not with low-dose and medium-dose ICS. As a result of sensitivity analyses, it was concluded that ICS had no adverse effects and can be explained by the severity of the disease at birth. We await further evidence regarding the effect of ICS on COVID-19 patients, but encouragingly, there are no clear reports of worsening cases. Thus, we strongly anticipate the continuation of normal treatment.

GINA, the most widely followed guideline worldwide, created a special chapter of interim guidance on asthma management during the COVID-19 pandemic in their 2020 December update (*29*) and posted the following guidance:

i) Advise patients to continue taking their prescribed asthma medications, particularly ICS. For patients with severe asthma, continue biologic therapy or oral corticosteroids if prescribed.

ii) Make sure that all patients have a written asthma action plan. The action plan tells the patient how to recognize worsening asthma, how to increase their reliever and controller medications, and when to seek medical help. Take a short course of oral corticosteroids when appropriate for severe asthma exacerbations.

iii) Where possible, avoid nebulizers due to the risk of spreading virus. A pressurized metered-dose inhaler *via* spacer is preferred except for life-threatening exacerbations. Add a mouthpiece or mask to the spacer if required.

iv) Avoid spirometry in patients with confirmed or suspected COVID-19, or if community transmission of COVID-19 is occurring in your region. Follow strict infection control procedures if aerosol-generating procedures such as nebulization, sputum induction, oxygen therapy and non-invasive ventilation are needed.

The Japanese society of Allergology makes almost the same recommendations in this context.

Molecular-targeted therapy with biologics is also an important option in adult patients with severe asthma. It has been reported that in patients with COVID-19, cytokines such as IL-4 increase (or remain the same), IL-5 remains the same, and IL-13 increases (*30*). Recently, there have been case reports of severe asthma patients diagnosed with COVID-19 and treated with an anti-IgE antibody (omalizumab) (*31*), an anti-IL-5 receptor antibody (benralizumab) (*32,33*), or an anti-IL-4α receptor antibody (dupilmab) (*34*), but had a course that eventually tested negative without exacerbation of asthma or pneumonia. Based on this, it is not necessary to cease treatment with biologic agents in asthma patients suffering from COVID-19. At our hospital, about 10% of asthma patients undergoing outpatient treatment are severe cases who use biologic agents, but none have COVID-19. Regarding the use of biologics in patients with severe asthma, The American Academy of Allergy, Asthma & Immunology (AAAAI) and the World Allergy Organization (WAO) recommended continuation during the COVID-19 pandemic and noted: "There is no evidence that the drug is harmful to COVID-19, and there is concern that asthma control may worsen due to discontinuation" (*35*).

Regarding ICS, the Japanese Society of Infectious Diseases is leading the observational study of the administration of ciclesonide that has been found to have an antiviral effect on SARS-CoV-2. At the same time, a multicenter, open-label, randomized trial, led by the National Center for Global Health and Medicine, is underway to investigate its efficacy and safety in treating asymptomatic and mildly ill COVID-19 in patients (*36*).

In conclusion, it could be speculated that asthma patients are not only unsusceptible to COVID-19, but also they are not aggravating factors for COVID-19, whereas having moderate-to-severe asthma might increase risk for severe illness from COVID-19. Because clear evidence concerning asthma treatment during the COVID-19 pandemic is still lacking, regular long-term treatment such as ICS and biologics for severe patients should be continued.

Funding: This study was supported by Health and Labor Sciences Research Grants (19HA1003).

Conflict of Interest: MH has, within the last 24 months, received honoraria from AstraZeneca, Glaxo SmithKline, Novartis Pharma, and Boeringer Ingelheim for lectures. Other co-authors have no conflicts of interest to disclose.

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Received December 26, 2020; Revised February 10, 2021; Accepted February 24, 2021.

Released online in J-STAGE as advance publication March 9, 2021.

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Global Health & Medicine. 2021; 3(2):73-81.
ORIGINAL ARTICLE
DOI: 10.35772/ghm.2020.01068

Epidemiological correlation between COVID-19 epidemic and prevalence of α-1 antitrypsin deficiency in the world

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Abstract: Among 68 countries in the world, severity of the COVID-19 epidemic was correlated with the prevalence of α-1 antitrypsin (AAT) deficiency. For the severe variant, PI*Z, the correlation coefficient (CC) was 0.8584 for the number of patients and 0.8713 for the number of deaths. For the milder variant, PI*S, it was 0.5818 and 0.6326, respectively. In Japan, the number of patients and deaths correlated with the population size with a CC of 0.6667 and 0.7074 respectively, and was proportional to the population size to the power of 1.65 and 1.54. The prevalence of AAT deficiency also correlated with the epidemiological pattern of COVID-19. In countries with high prevalence of AAT deficiency, after the initial rise, the daily number of patients and that of deaths ran parallel at a high level for more than 6 months without sign of abatement. In countries with a low prevalence of AAT deficiency, after the first wave of the epidemic, the number of the deaths decreased continuously while the number of patients remained the same or even increased resulting in a decreasing case-fatality rate. When the cumulative number of deaths was plotted on the y-axis against the cumulative number of patients on the x-axis, plots fell on a straight line in countries with a high prevalence of AAT deficiency; while in countries with a low prevalence of AAT deficiency, a break appeared, after which the plots fell on flatter slope indicating decreasing case-fatality rate. The observation suggests emergence of an attenuated variant in countries with a low prevalence of AAT deficiency.

***Keywords*:** COVID-19, coronavirus, adaptation, quasi-species, case-fatality

Introduction

I was interested in why damage caused by COVID-19, epidemic of SARS-CoV-2, was so severe in countries in European and American continents despite of their advanced public health. I preliminarily examined the relation between COVID-19 and α-1 antitrypsin (AAT) deficiency, "a genetic disorder predominantly arising in those in European stock" according to Hutchinson (*1*). In the analysis, I used statistics on AAT deficiency published by de Serres *et al.* in 2012 (*2*). The number of the patients and that of the deaths (as of 19 May 2020) were correlated with the number of people with the more severe variant PI*Z of AAT deficiency with correlation coefficient (CC) 0.6049 and 0.6721, respectively. The correlation with the milder variant, PI*S, was 0.4207 and 0.4660 for patients and deaths respectively.

I recently found that Blanco *et al.* published new AAT deficiency statistics in 2017 (*3*). As the analysis strongly depends on the statistics of AAT deficiency, I reinvestigated the issue using the Blanco *et al.*'s statistics and the SARS-COV-2 data updated on 15 June 2020. I found the number of infections and that of the deaths due to SARS-CoV-2 infection were correlated with the prevalence of AAT deficiency with correlation coefficients of 0.8584 and 0.8713 for variant PI*Z, and 0.6326 and 0.5818 for PI*S, confirming my previous analysis.

The clinical manifestations of AAT deficiency varies widely from asymptomatic to fatal liver or lung diseases. As features suspicious of AAT deficiency, American Thoracic Society and the European Respiratory Society (ATS/ERS) counted early onset of emphysema (age of 45 years or less), emphysema in the absence of a recognized risk factor, emphysema with prominent basilar hyperlucency, and otherwise unexpected liver disease, *etc.* (*4*). For the clinical manifestation, tobacco smoking, exhaust gas, exposure to pathogens, *etc.* were known to be involved (*4*). Thus, AAT deficiency is "not a rare disease but a disease that is rarely diagnosed" (*5*).

AAT is a 52-kDa protein encoded by *SERPINA1* gene located in the 14th chromosome. The normal allele is coded as PI*M. The most frequent mutant alleles are PI*S and PI*Z, among which the deficiency was more severe for the latter. AAT is secreted from liver, and the protein encoded by the mutant alleles forms a polymer that is retained within hepatocytes resulting in the reduced serum level of AAT (*3*). According to the joint statement of ATS/ERS (*4*), the serum level (mg/dL) of AAT was 150-350 for PI*MM homozygotes (normal

individuals), 90-210 for PI*MZ heterozygotes, 100-200 for PI*SS homozygotes, 75-120 for PI*SZ heterozygotes, and 20-45 for PI*ZZ homozygotes. Estimated gene frequency per 1,000 population in Europe and the United States were respectively 1-9 and 2-4 for PI*S, and 2-24 and 1-2 for PI*Z (*4*). In Japan, AAT deficiency is listed among the "intractable diseases" (*6*), but the prevalence is < 1/1,000 population (*3*).

AAT counterbalances neutrophil elastase and other serine proteinases including trypsin. As we did not know which specific protease(s) are counterbalanced by anti-trypsin in the context of SARS-CoV-2 infection, protease(s) counterbalanced by anti-trypsin will be simply referred to as trypsin in this article.

The present study revealed an unexpected epidemiological correlation between the COVID-19 epidemic and AAT deficiency. The epidemiological data of COVID-19 used in this report were those from 21 January (report number 1, Rp1) to 26 June of 2020 (Rp158). As the COVID-19 pandemic progresses, new epidemiological features may emerge.

Materials and Methods

The COVID-19 epidemic data were derived from WHO Coronavirus disease (COVID-19) situation reports (*https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports*) issued from 21 January 2020 to 18 June 2020, and the AAT deficiency prevalence data from tables published by Blanco *et al.* (*2*) (Table 1; Table S1-S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*). The population size and population density data were derived from *https://www.worldometers.info/world-population/population-by-country* and *https://worldpopulationreview.com/country-rankings/countries-by-density* both downloaded on 20 July 2020. Age distribution of countries were derived from *https://data.worldbank.org/indicator/SP.POP.65UP.TO.ZS* downloaded on 20 July 2020.

Results

Correlation between AAT deficiency and COVID-19 morbidity and mortality

Table 1 lists the correlation coefficients (CC) between the number of people with AAT deficiency and the number of patients and deaths due to SARS-CoV-2 infection. The third column lists CCs for all analyzed countries combined. The fourth column CCs are for European and American countries, where 44 of 44 countries have a population with AAT deficiency. The fifth column CCs are for the remaining countries in other regions combined, of which 14 in 24 countries have a population with AAT deficiency variant PI*S, and 9 in 24 have a population with AAT deficiency variant PI*Z.

For all the countries combined, CC between the number of patients and the number of people with AAT deficiency PI*S, PI*Z or PI*SZ was 0.5818, 0.8584, and 0.7393, respectively, and CC between the number of deaths and the number of people with AAT deficiency was 0.6326, 0.8713, or 0.8585, respectively. When American and European countries were combined, excluding countries in the other regions, CC between the number of patients and the number of people with AAT deficiency PI*S, PI*Z or PI*SZ increased to 0.7594, 0.9170 and 0.7656, respectively; and CC between the number of deaths and the number of people with AAT deficiency PI*S, PI*Z or PI*SZ increased to 0.8244, 0.9503, and 0.8864. For the other regions however, CCs between the number of patients or deaths and the number of people with AAT deficiency became

Table 1. Correlation coeficients between COVI D-19 Morbidity or Mortality and Population with AAT Deficiency

AAT Deficiency	COVID-19	All (A + B)	Europe and America (A)	Other Regions (B)
PI*S	Patients	0.5818	0.7594	0.6670
	Deaths	0.6326	0.8244	0.4360
PI*Z	Patients	0.8584	0.9170	0.5697
	Deaths	0.8713	0.9503	0.4360
PI*SZ	Patients	0.7393	0.7656	0.0925
	Deaths	0.8585	0.8864	0.0253
Population size	Patients	0.3050	0.8941	0.6667
	Deaths	0.1701	0.8417	0.7074
> 65 years	Patients	0.3050	0.9038	0.4560
	Deaths	0.2701	0.8894	0.5403

European countries: Denmark, Estonia, Finland, Iceland, Latvia, Lithuania, Norway, Sweden, Belgium, France, Netherlands, Rep Ireland, United Kingdom, Austria, Germany, Poland, Switzerland, Italy, Portugal, Spain, North Macedonia, Russian Federation, Serbia.
American countries: Canada, USA, Mexico, Costa Rica, Cuba, Dominican Republic, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Argentina, Bolivia, Brazil, Chile, Columbia, Ecuador, Paraguay, Peru, Uruguay, Venezuela.
African countries: Cameroon, Cape Verde, Morocco, Nigeria, Somalia, Tunisia, Democratic Republic Congo, Mozambique, Republic Congo, South Africa.
Asian countries: Kazakhstan, China, Indonesia, Japan, Malaysia, Mongolia, Papua New Guinea, Philippines, Singapore, Republic Korea, Thailand, Nepal, Pakistan, India.
Underlined countries are those with < 1/1,000 incidence both for PI*S and PI*Z.

almost insignificant.

Interestingly however, within each region, CC between the population size and the number of patients and number of deaths increased both for European and American countries and for countries in the other regions. European and American countries' CC was 0.8941 for the patients and 0.8417 for the deaths; and for countries in the other regions, CC was 0.6667 for the patients and 0.7074 for the deaths.

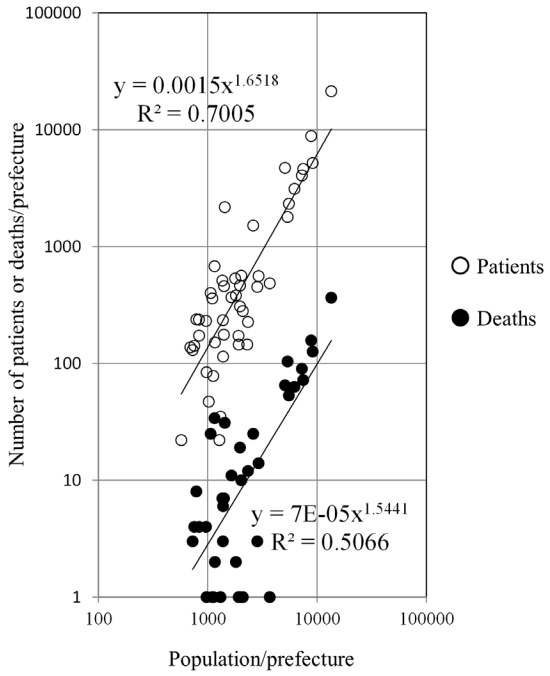

Figure 1. Relation between the number of the patients or the deaths due to SARS-COV-2 infection and the population size among 47 prefectures in Japan. The number of patients (○) or number of deaths (●) is plotted on the vertical axis and the population size of prefectures on the horizontal axis. Both axes are logarithmic scales. Prefectures with zero deaths were excluded from the plot for the deaths. CC was 0.8778 for the patients and 0.9062 for the deaths.

The above observation led me to suspect that infection of SARS-CoV-2 and accompanying deaths occurred almost at random within each group of the countries. Therefore I calculated the CC between the number of the SARS-CoV-2 patients or deaths (published in a daily newspaper Mainichi Shimbun, morning edition, on 4 September) and the population size among prefectures in Japan (Statistic bureau of Japan, *https://www.stat.go.jp/data/nihon/02.html*). CC between the number of patients and the population size was 0.8778, and CC between the number of deaths and the population size was 0.9062. I then plotted, on a logarithmic scale, the number of patients or deaths on the y-axis and the number of people on the x- axis for 47 prefectures in Japan. As shown in Figure 1, the relation between the number of patients (○) and the population size is represented by equation $y = 0.0015x^{1.652}$ with $R^2 = 0.7005$, and the relation between the number of deaths (●) and the population size by equation $y = 7E\text{-}05x^{1.544}$ with $R^2 = 0.5066$. The slope of the plots for COVID-19 is almost the same as for the measles epidemic (*7*), indicating that the COVID-19 epidemic is dependent on population size in the same way as the measles epidemic.

Figure 2 shows plots of the number of patients on the y-axis against the number of people with AAT deficiency variant PI*S (panel A), variant PI*Z (panel B) or the total population (panel C) on the x-axis for countries with AAT deficiency (> 1/1,000 population), which were mostly European and American countries.

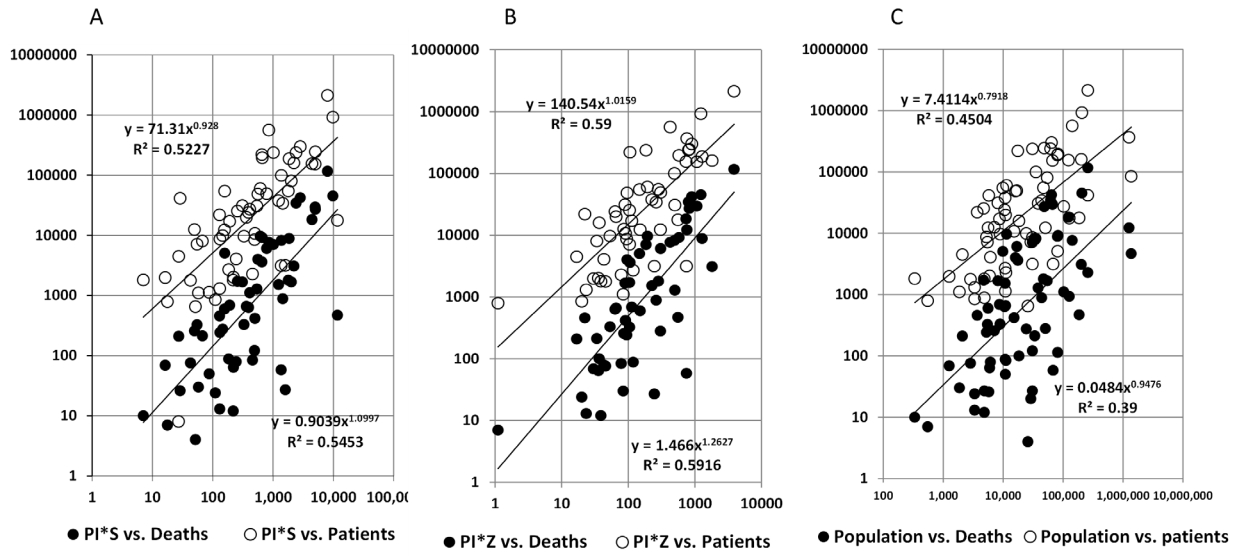

Figure 2. Relation between the number of the patients (○) or deaths (●) due to SARS-CoV-2 infection and the AAT deficient population among countries, panel A for variant PI*S, and panel B for variant PI*Z. Panel C shows the relation between the number of patients (○) or deaths (●) due to SARS-CoV-2 infection and population size (x1,000). The number of patients (○) or deaths (●) is plotted in the vertical axis and the population size of countries (x1,000) in the horizontal axis, both in the logarithmic scale. Countries with prevalence of AAT deficiency < 1/1,000 were excluded from this analysis.

The relation between the number of COVID-19 patients (y) and the AAT deficiency population (x) was expressed by equations $y = 140.54x^{1.02}$ with $R^2 = 0.59$ for PI*Z and $y = 71.31x^{0.93}$ with $R^2 = 0.52$ for PI*S; the relation between the number of COVID-19 deaths (y) and the AAT deficiency population (x) by equations $y = 1.466x^{1.26}$ with $R^2 = 0.59$ for PI*Z and $y = 0.9039x^{1.10}$ with $R^2 = 0.55$ for PI*S.

Epidemic curve of COVID-19

Figure 3A and B show plots of the daily number of new patients (open symbols) and new deaths (closed symbols) on a logarithmic scale from 21 January 2020 (Rp1 the first WHO situation report) to 26 June (Rp158). Also plotted, on a logarithmic scale, are the daily number of new deaths (D) divided by new infections (P), D/P, which is found in the area $y < 1$. Here, D/P is a parameter for monitoring the trends of new deaths relative to new infections. Curves obtained by the above plots will be called epidemic curves.

Through inspection of the epidemic curves, an epidemic model was constructed (see Table S3A for tabulation, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*). In the model (Figure 5A), the plot for the number of patients and deaths are wave shaped; the peak of the deaths (●) is found on the right side of that of the patients (○). D/P decreases continuously until the number of patients reaches a peak; after that, if the number of patients (○) and deaths (●) decline, D/P increases towards the end of the epidemic (Δ); if the new infection stops decreasing in the middle (□) while the number of deaths continuously decreases (●), D/P declines again (◊).

With this model, countries fell into two groups. One is represented typically by China (Figure 3A). For China, there were three waves for patients, W1, W2 and W3, and one wave for deaths that dragged on from W1 (panel A in Figure 3A). For Rep Korea and Japan, there were two waves of patients, W1 and W2, and one wave of the deaths (panels B and C). D/P increased continuously until the transition between W1 and W2 (marked by a downward arrow) and then declined. Singapore and Malaysia (panel D) exhibited a similar pattern except the first wave of patients dragged on and the number of deaths declined continuously; as a consequence, D/P continuously decreased and faded away. The above countries all have a low prevalence of AAT deficiency. This plot pattern was shared by Morocco (prevalence of PI*S at 67/1,000 and that of PI*Z at 34/1,000) (*3*)) (panel E), United Arab Emirates (UAE) (prevalence of AAT deficiency unknown) (panel E), Cameroon (prevalence of PI*S at 146/1,000 and PI*Z at < 1/1,000) (*3*)) (panel F), DR Congo (prevalence of PI*S or PI*Z at < 1/1,000) (*3*)) (panel F), Kenya (prevalence of AAT deficiency unknown) (panel G) and Niger (prevalence of AAT deficiency unknown) (panels G).

Plots for Australia and New Zealand (panel H) were characterized by a pyramid-shaped wave of the number of patients and low number of deaths; D/P decreased first and then gradually increased and faded away. Australia and New Zealand are countries with a relatively high prevalence of AAT deficiency (PI*Z frequency 12.2/1,000 for Australia and 26/1,000 for New Zealand; PI*S frequency 42.2/1,000 for Australia and 33/1,000 for New Zealand) (*2*)).

The plot pattern was entirely different for countries with a high prevalence of AAT deficiency (Figure 3B). This pattern was shared by Sweden, Germany, Switzerland, Italy, Spain, United Kingdom (UK), Belarus, Russian Federation, France and Belgium in Europe (panels I-L); USA, Canada, Mexico, Peru, Chile, and Brazil in the Americas (panels M-N); and India, Bangladesh, Indonesia and Philippines (panels O-P). Among them, the countries in European and American continents have a high prevalence of PI*S, PI*Z or both (Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*). Among the other countries, India has a high prevalence of PI*Z, Philippines has high prevalence of PI*S and Indonesia has prevalence < 1/1,000 both for PI*S and PI*Z (Table S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*). Prevalence of AAT deficiency in Bangladesh is unknown.

Case vs. fatality plot

In Figure 4, the cumulative number of patients is plotted on the x-axis against the cumulative number of deaths on the y-axis, both on a logarithmic scale. Here, the plot starts from the date when the cumulative number of deaths exceeded 10 and started increasing continuously, because, in my preliminary study, I found that the number of deaths fluctuated aberrantly in the initial phase. Therefore, time range is indicated by the report number together with the country name, such as, China Rp40-87.

In principle, the case-fatality rate (CFR) does not change for a fixed pathogen and host pair. Therefore, the plot should expectedly fall on a straight line with slope of 1. This was not the case for many of the countries, however. For USA, the plot has a slope of 1.76, *i.e.*, the CFR increased continuously as the epidemic progressed (Figure 4D). For China, there was a break, after which the plot becomes flatter, *i.e.*, the CFR decreased progressively thereafter (Figure 4A). The virulence of viruses or the susceptibility of hosts does not change in such ways: what occurs is random mutation and selection. The above phenomena have to be explained in that context. "Two-population model" was developed for this purpose (*8*).

Simulation of the plot with a steep inclination angle (> 45°) is shown in Figure 5B and tabulation of the simulation in Table S3B (*https://www.*

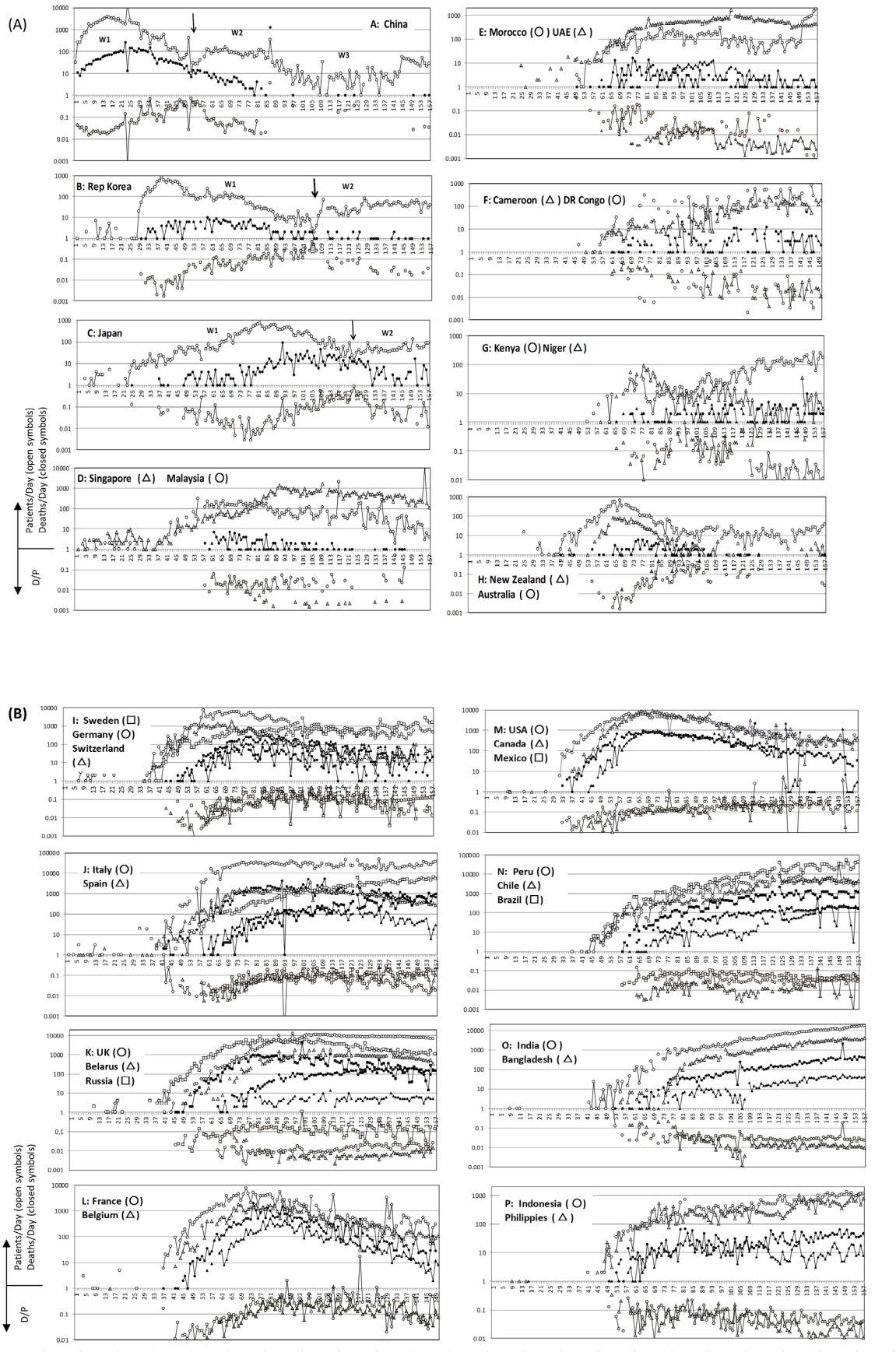

Figure 3. A and B, Epidemiological curves of COVID-19 for countries in various regions in the world. Daily incidence of new patients (open symbols) and new deaths (closed symbols) are plotted in the area y > 1; D/P (deaths/patient) is plotted in larger open symbols in the area y < 1.

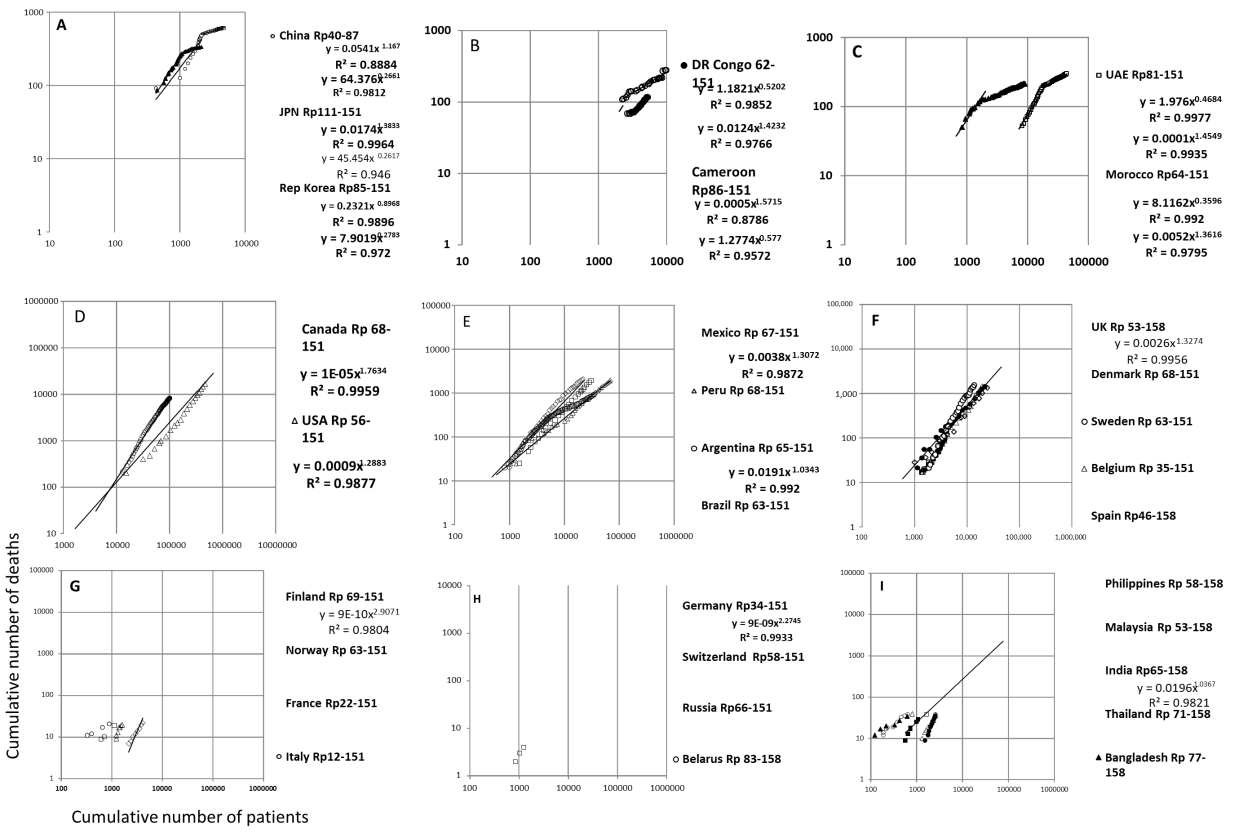

Figure 4. Case-fatality plots for various countries. The cumulative number of deaths is plotted on the vertical axis and cumulative number of patients on the horizontal axis, both on a logarithmic scale. The plot range is indicated by annotation, *e.g.*, Rp40-87 meaning that the plot range was from the WHO report number 40 to 87.

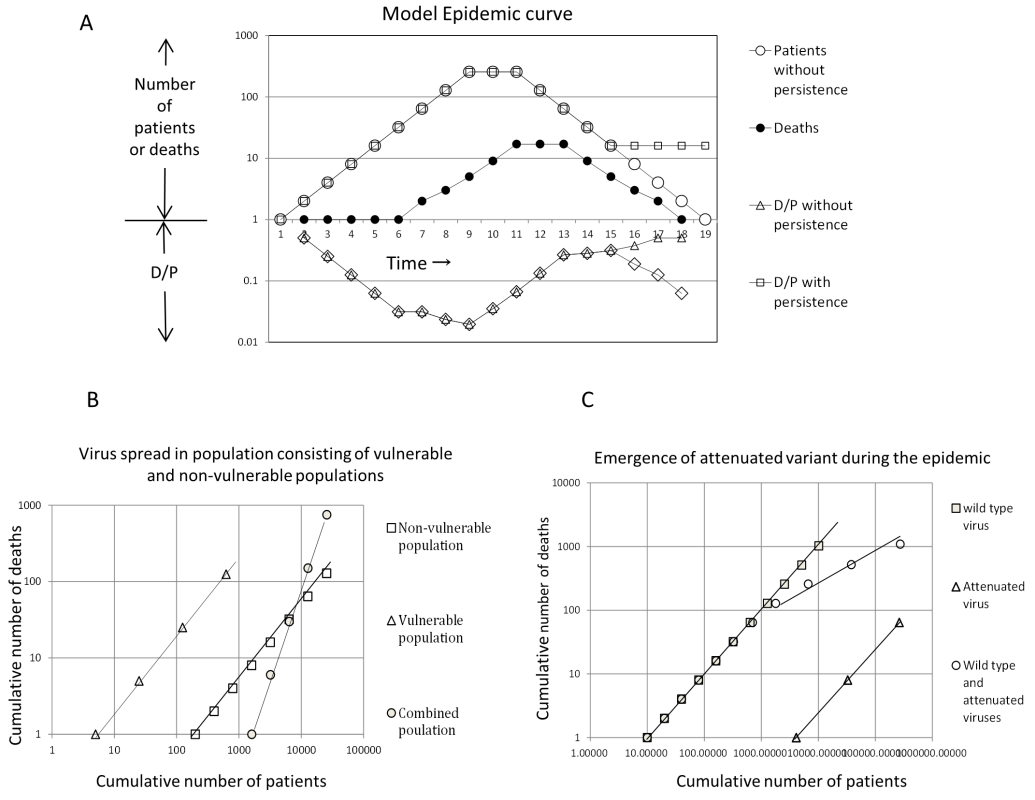

Figure 5. Model epidemic curve (A), case-fatality plot simulated for propagation of the virus in population consisting of vulnerable and non-vulnerable population (B), and simulated case-fatality plot for epidemic with emergent attenuated variant (C). Tabulation for the plot in panel A is found in Table S3A, that for panel B in Table S3B, and that for panel C in Table S3C. Explanations of symbols are found on the right side of the figures. See text for other details.

globalhealthmedicine.com/site/supplementaldata.html?ID=9). The model assumes that the population consists of a vulnerable minor subset (Δ), such as aged people in nursing homes, and a non-vulnerable major population (□); CFR is 1/5 for the former and 1/200 in the latter; and the speed of the spread is 2.5-fold more rapid for the former than for the latter. The 'Combined' column in Table S3B (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*) is the number of patients or deaths in the vulnerable population and those of the non-vulnerable population added together. Plot of the added number of the patients on the x-axis against the added number of deaths on the y-axis both on a logarithmic scale (○) falls on a straight line with a slope of 1.4, which matches the steep slope plots for USA, Canada, Germany, Switzerland, *etc.* (Figure. 4D, 4F-4H); they are countries with a high population of elderly (population > 65 years was 16% for USA, 18% for Canada, and 22% for Germany). For Philippines, Malaysia, India, Thailand and Bangladesh (Figure 4I), Argentina and other countries in South America (Figure 4E) and Belarus (Figure 4H), the slope was near 1. These countries had a younger population (population > 65 years was 5% for Philippines and Bangladesh, 6% for India, 7% for Malaysia, 11% for Argentina, 12% for Thailand, and 15% for Belarus).

Simulation for the plot with a break is shown in Figure 5C and tabulation of the simulation in Table S3C (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*). The simulation assumes that CFR of the wild type is 1/10 and that of the attenuated variant 1/4,096; in the middle of the epidemic (time 9 in Table S3C, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*) of the wild type (□), the attenuated variant (Δ) emerges and spreads 4-fold more rapidly than the wild type. For the patients and deaths, the number for the wild type and the attenuated mutant are added together. The case-fatality plot for the two virus populations combined (○) initially has a slope of 1.0 but after the break its slope becomes 0.4. The model fits well with the plots for China, Rep Korea, and Japan, DR Congo, Cameroon, UAE and Morocco (Figure 4A-C).

Discussion

Among the reported countries, the number of patients and number of deaths due to SARS-CoV-2 infection correlate with the prevalence of variant PI*Z of AAT deficiency with a CC of 0.8584 and 0.8713, respectively. Correlation of the number of patients or deaths with the prevalence of PI*S is lower than that of PI*Z, which is reasonable because the serum level of α-1 antitrypsin is lower for PI*Z than for PI*S (AAT level relative to PI*MM was ~80% for PI*MS, 60% for PI*SS, 55% for PI*MZ, 40% for PI*SZ, and 15% for PI*ZZ (*2*)). Though AAT is an acute phase reactant, the pleomorphism of AAT was reflected in the normal time plasma level (*9*). Thus, the normal time plasma level should have strongly affected the outcome of SARS-CoV-2 infection. Recently Vianello and Braccioni reported geographical overlap between α-1 antitrypsin deficiency and SARS-CoV-2 infection in Italy (*10*).

The high correlation between the number of patients and deaths due to SARS-CoV-2 infection and the prevalence of AAT deficiency is equivalent to say that the propagation and pathogenicity of SARS-CoV-2 depends on exogenous trypsin, because, in people with a normal level of α1-antitrypsin, the level of active trypsin is kept in check by α1-antitrypsin, and viruses requiring trypsin remain under control, but in people with AAT deficiency, the trypsin level is not suppressed allowing for a high amount of trypsin to be available for the virus. Here, I used the term trypsin, but it could be other serine proteases, notably neutrophil elastase.

An important question is when and where the virus acquired property of trypsin dependency. In my own experience, trypsin-dependency emerged among mouse hepatitis virus released from normal looking carrier cells as an attenuated variant requiring trypsin (*11*) or coinfection with mouse leukemia virus (*12*) for plaque formation. Trypsin dependency will endow the virus with an increased chance to spread in populations with normal levels of AAT, because, although the replication of the virus in infected people may be slowed, the chance of the virus to spread will increase as infected people remain asymptomatic: it was reported that people asymptomatically infected by SARS-CoV-2 shed virus significantly longer than symptomatically infected patients (*13*). Therefore, I speculate that the trypsin dependency emerged as a process of adaptation to humans. Recently however, Wichit *et al.* reported that clinical isolate of porcine endemic diarrhea coronavirus required supplementation of exogenous trypsin (*14*). They argued that it was brought about by confinement of the natural infection of the virus in the protease-rich small intestine of pigs. Menachery *et al.* reported that a SARS-like coronavirus that bats harbor had the ability to infect humans without adaptation, but the virus needed exogenous protease treatment for isolation (*15*). Therefore, it is possible that SARS-CoV-2 was dependent on the exogenous trypsin before it was introduced into the human community.

In countries with a low prevalence of AAT deficiency, SARS-CoV-2 must have been experiencing attenuation because, in the case-fatality plots for China, Rep. Korea and Japan, there emerged a break followed by flatter plot resulting in decreasing fatality (Figure 4A). For China, the break was at Rp58 on 18 March 2020, and for Japan, it was at Rp131 on 29 May. Such a trend was also observed among DR Congo, Cameroon, UAE, and Morocco. They are not necessarily countries with a low prevalence of AAT deficiency, however.

The mutation involved in the attenuation could be

deletion in ORF8 observed among attenuated SARS virus in 2008 (*16*) or deletion in ORF3 for attenuated Middle East Respiratory Syndrome (*17*). The attenuated SARS mutant had reduced replication capacity, and could be recovered only by the reverse-genetics (*16*). It appeared that the mutant had an advantage in propagation on account of the reduced pathogenicity that permitted persistence in the host (*16*). It should be recalled that persistence is one of the important characteristics for successful propagation of viruses among hosts (*18*). Recently, attenuated variants of SARS-CoV-2 were obtained, which had a deletion in ORF7b and ORF8 (*19,20*) or in S1/S2 junction (*21*).

Though the above data suggests a strong correlation between the severity of the epidemic and the prevalence of AAT deficiency, there are some exceptions. For Indonesia, though AAT deficiency prevalence is < 1/1,000 (*3*), the number of patients and deaths were 41,431 and 2,276, respectively (Table S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*). For Morocco, while the epidemiological curve resembled that of countries with a low prevalence of AAT deficiency, the prevalence of AAT deficiency was actually high (34/1,000 for PI*Z) and the number of patients and deaths were 8,020 and 213 respectively. Other exceptions were Australia and New Zealand: though the prevalence of AAT deficiency was high, the epidemiological curve resembled that of countries with low prevalence of AAT deficiency. In these countries, small population size and low population density may have played a role (for Australia, population size is 25,499,884 (55^{th} in the world) and population density 3/km sq. (226^{th} in the world); for New Zealand population size is 4,822,233 (126^{th} in the world) and population density is 18/km² (200^{th} in the world)). For spread of the virus in such a geographical environment, the mobility of infected people should have been critically important, and variants with lower virulence could have been selected for. Further exploration of the exceptional cases will lead to a better understanding of the relation between COVID-19 epidemics and AAT deficiency.

An important question not addressed above is the risk of COVID-19 deaths among populations with AAT deficiency relative to the risk among populations without. To answer this question, we have to know the frequency of AAT deficiency among COVID-19 patients and COVID-19 deaths. As there is currently no such information, it is not possible to answer this question. However, the following assessment could be possible. For example, if we suppose that the number of COVID-19 deaths per population in countries without AAT deficiency reflects the risk of deaths among the population without, it could be at most 0.074 ‰ (see column C5:D/Pop (‰) for Japan in Table S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*). As "D/Pop (‰)" in Portugal with the highest prevalence of AATD was 1.405 ‰ (Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=9*), the relative risk could be obtained by dividing 1.405‰ by 0.074‰, *i.e.*, 18.9-fold. As individuals with AAT deficiency occupied only 13.5% of the population in Portugal, the relative risk of the population with AAT deficiency could have been higher than that value. If all the fatality due to the SARS-CoV-2 infection was borne by the population with AAT deficiency in Portugal, relative risk could be calculated by dividing 18.9 by 0.135 to obtain 140-fold.

In conclusion, the COVID-19 epidemic was found to be under the influence of AAT deficiency globally, but within a region or in a country it depends on population size. It is important to note that the severity of the epidemic was influenced by other factors (*22-23*). The COVID-19 epidemic is still progressing as of early September 2020. The epidemiology of COVID-19 needs to be followed closely.

Acknowledgements

I acknowledge that the present study could not have been initiated without the data on prevalence of AAT deficiency compiled by F.J. Serres and I. Blanco. I thank Prof. Ignacio Blanco, Fundacion Registro Espanol del DAAT and Prof. Andrea Vianello, University of Padua for information and advice. I thank AIDS Research Center, National Institute of Infectious Disease for providing me with the space to conduct this research.

Funding: None.

Conflict of Interest: The author has no conflict of interest to disclose.

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Received August 2, 2020; Revised September 7, 2020; Accepted September 15, 2020.

Released online in J-STAGE as advance publication October 12, 2020.

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Global Health & Medicine. 2021; 3(2):82-89.
ORIGINAL ARTICLE
DOI: 10.35772/ghm.2020.01102

Characteristics of patients with novel coronavirus disease (COVID-19) during the first surge versus the second surge of infections in Osaka Prefecture, Japan

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Abstract: In Japan, the differences in characteristics, severity, and mortality of novel coronavirus disease (COVID-19) patients between the first and second surges of infections have not been fully understood. This study is a retrospective cohort study of COVID-19 patients confirmed between February 1 and August 31, 2020 in Osaka Prefecture, Japan. Publicly available information on patients was collected from the website of Osaka Prefecture. Patients were divided into two groups according to the date of the positive laboratory test result: the first surge (February 1 to May 22) and the second surge (May 23 to August 31). Patients' characteristics were compared between the two groups. A multivariable Cox proportional-hazards model was applied to compare severity and mortality between the two groups, where sex, age group at the onset date, city of residence, and days to test positive were adjusted. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. A total of 8,541 patients included 1,780 and 6,761 patients in the first and second surges, respectively. Age at the onset date was younger in the second surge ($p < 0.001$), and median of days from the onset date to the positive test date shortened from 7 to 6 days ($p < 0.001$). The multivariable Cox proportional-hazards model revealed that both severity and mortality were lower in the second surge than in the first surge (severity: HR: 0.51 [0.39-0.67]; mortality: HR: 0.37 [0.25-0.56]). In conclusion, severity and mortality were lower in the second surge than in the first surge among COVID-19 patients in Osaka Prefecture, Japan.

***Keywords*:** SARS-CoV-2, severity, mortality, survival analysis

Introduction

The novel coronavirus disease 2019 (COVID-19), which was identified in Wuhan, China in December 2019, has spread to countries all over the world (*1-3*). In Japan, the cumulative number of patients was 67,865 on August 31, 2020 (*4*). The daily number of newly diagnosed patients has changed over time. The first COVID-19 case in Japan was identified on January 16, 2020, and subsequently, the daily number of patients began to rise (the first surge), and reached its first peak on April 10, 2020 (*4*). Although the daily number of patients decreased after the initial peak, it started to increase again since the middle of June (the second surge), and reached its second peak on August 7, 2020 (*4*). Although the daily number of patients decreased after the second peak, it started to increase again in October. As of December 2020, Japan is in the middle of the third surge of infections (*4*). Although a prior study (*5*) compared the characteristics of COVID-19 patients between the first and second surges in the United States, little is known about the differences in characteristics, severity, and mortality of COVID-19 patients between the first and second surges.

Osaka Prefecture is the largest metropolitan area in the western part of Japan, with a population of 8.8 million, and a total area of 1,905 km^2 (*6*). It has the second highest number of COVID-19 patients in Japan next to Tokyo (*4,6*). The present study aimed to assess the difference in characteristics, severity, and mortality of COVID-19 patients between the first and the second surges of infections in Osaka Prefecture, Japan.

Methods

Study design

This study was a retrospective observational study conducted from February 1 to August 31, 2020. Publicly available data on laboratory-confirmed COVID-19 patients in Osaka Prefecture, Japan, were collected from the Osaka Prefecture website (*7*). Since we only collected anonymous data, the necessity of obtaining informed consent from the participants was waived (*6*).

This study was approved by the Ethics Committee of Osaka University Graduate School of Medicine (approval No. 20089).

Official data collection of COVID-19 patients in Osaka Prefecture

Details on the data collection have been previously published elsewhere (*6*) and are available on the Osaka Prefecture website (*7*). In brief, the following information on COVID-19 patients is available on the website of Osaka Prefecture: sex, age group at the onset date, city of residence, onset date, date when the patient showed positive results in the laboratory test, date when a patient's condition became severe (for patients whose condition turned severe during the observation period), and date of death (for patients who died during the observation period) (*6,7*). We defined onset date as the date when any symptoms appeared (*6*); onset date was missing for patients who did not present any symptoms. The date when a patient's condition became severe was defined as the date when a patient met any of the following criteria: *i*) received mechanical ventilation, *ii*) received extracorporeal membrane oxygenation (ECMO), and *iii*) was admitted to the intensive care unit (ICU) (*8*).

Definition of group

We categorized COVID-19 patients in Osaka Prefecture who were confirmed as COVID-19 between February 1 and August 31 into two groups according to the date of the positive laboratory test result: patients who showed positive results in the laboratory test between February 1, 2020 and May 22, 2020 (the first surge) and patients who showed positive results in the laboratory test between May 23, 2020 to August 31, 2020 (the second surge). The Japanese government declared a state of emergency in Osaka Prefecture on April 7, 2020, and it lifted the state of emergency in Osaka Prefecture on May 21, 2020 (*9*). The end of the first surge was defined based on the following two reasons: *i*) patients whose samples were submitted for laboratory tests on May 21 (the end of the state of emergency) were reported on May 22; and *ii*) the number of newly diagnosed COVID-19 patients was zero on May 23 for the first time. The end of the second surge in this study was defined as the final day of the present study.

Endpoints

The outcome measures in this study were increased disease severity, and all-causes of mortality. All patients were followed-up for 30 days from the onset date.

Statistical analyses

The baseline characteristics of COVID-19 patients were compared between the first and the second surges. Sex, age group at the onset date (0-59, 60-69, 70-79, 80-89, ≥ 90 years), and city of residence (Osaka City, other cities, unknown) were compared between the two groups using the chi-square test. Days from the onset date to the date when the patient showed positive results in the laboratory test (days to test positive) were summarized by interquartile range in each group, and they were compared between the two groups by applying the Wilcoxon rank sum test. We also categorized days to test positive by quartile, and analyzed the distribution of age groups according to the four categories.

We compared the severity of the condition of COVID-19 patients between the first and the second surges. Patients with missing information on the onset date or the date when the patient's condition became severe (for patients whose condition became severe during the observation period) were excluded from this analysis. The Kaplan-Meier method was applied to compare the cumulative probability of developing severe disease stratified by age group at the onset date (aged ≤ 69 years or ≥ 70 years), and the difference was compared between the first and the second surges by the log-rank test. The multivariable Cox proportional-hazards model was applied to compare disease severity between the two groups, where sex, age group at the onset date (0-59, 60-69, 70-79, 80-89, ≥ 90 years, unknown), city of residence (Osaka City, other cities, unknown), and days to test positive (categorized by quartile) were adjusted. We also stratified the study population according to age group at the onset date (aged ≤ 69 years or ≥ 70 years), and applied the multivariable Cox proportional-hazards model in each stratum.

We also compared the mortality of COVID-19 patients between the first and the second surges. Patients with missing information on the onset date or the date of death (for patients who died during the observation period) were excluded from this analysis. The Kaplan-Meier method was applied to compare cumulative mortality stratified by age group at the onset date (aged ≤ 69 years or ≥ 70 years), and the difference was compared between the first and the second surges by the log-rank test. The multivariable Cox proportional-hazards model was applied to compare mortality between the two groups. Variables adjusted in this model were the same as those adjusted in the multivariable Cox proportional-hazards model when we compared severity between the two groups. We also stratified the study population according to age group at the onset date (aged ≤ 69 years or ≥ 70 years), and applied the multivariable Cox proportional-hazards model in each stratum.

All analyses in the present study were conducted using STATA version 16.0 MP software (StataCorp LP). All tests were two-tailed, and we considered p-values < 0.05 as statistically significant.

Results

A total of 8,541 patients were confirmed as COVID-19 positive between February 1 and August 31, 2020 in Osaka Prefecture, Japan. The first and second surges consisted of 1,780 and 6,761 patients, respectively. Figure 1 presents the number of laboratory tests and COVID-19 patients recorded per day in Osaka Prefecture, Japan. In the first surge, the daily number of newly diagnosed COVID-19 patients reached its peak on April 9 (92 cases per day). In the second surge, the daily number of newly diagnosed COVID-19 patients reached its peak on August 7 (255 cases per day).

Table 1 describes the characteristics of COVID-19 patients in the first and second surges. Although the difference in the distribution of sex was not statistically significant (p = 0.08), the proportion of male was higher in the second surge than in the first surge. The proportion of patients aged 0-59 years was higher in the second surge than in the first surge. The proportion of patients living in Osaka City was higher in the second surge than in the first surge. Days to test positive was significantly lower in the second surge than in the first surge (p < 0.001). Supplementary Table S1 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=11*) describes the distribution of age groups according to four categories (Q1-Q4) of days to test positive. The proportion of patients aged ≥ 70 years was highest in Q1 (15.9%), and lowest in Q3 (8.9%).

Figure 2 shows the Kaplan-Meier curves for disease severity among patients aged ≤ 69 years (Figure 2A) and ≥ 70 years (Figure 2B); 7,122 patients were included in the analysis of severity. The log-rank test revealed that severity was significantly lower in the second surge than in the first surge in each stratum (p < 0.001 and p = 0.03, for patients aged ≤ 69 years and ≥ 70 years, respectively). Table 2 describes the number of patients, number of events, and adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) by the multivariable Cox proportional-hazards model for severity. Severity was lower in the second surge than in the first surge (adjusted HR: 0.51, 95% CI: 0.39-0.67). In the stratified analysis, severity was lower in the second surge than in the first surge among both patients aged ≤ 69 years and those aged ≥ 70 years (HR: 0.51 and 0.64, for patients aged ≤ 69 years and ≥ 70 years, respectively). Supplementary Table S2 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=11*) describes HRs and 95% CIs for all the variables adjusted in the multivariable Cox regression analysis on severity. Higher severity was observed among male than among female (adjusted HR: 2.70, 95% CI: 2.01-3.65). Significantly higher severity was observed among patients aged ≥ 60 years compared with those aged ≤ 59 years, except for that among patients aged ≥ 90 years. Severity was similar regardless of the city of residence or days to test positive.

Figures 3(A) and 3(B) show the Kaplan-Meier

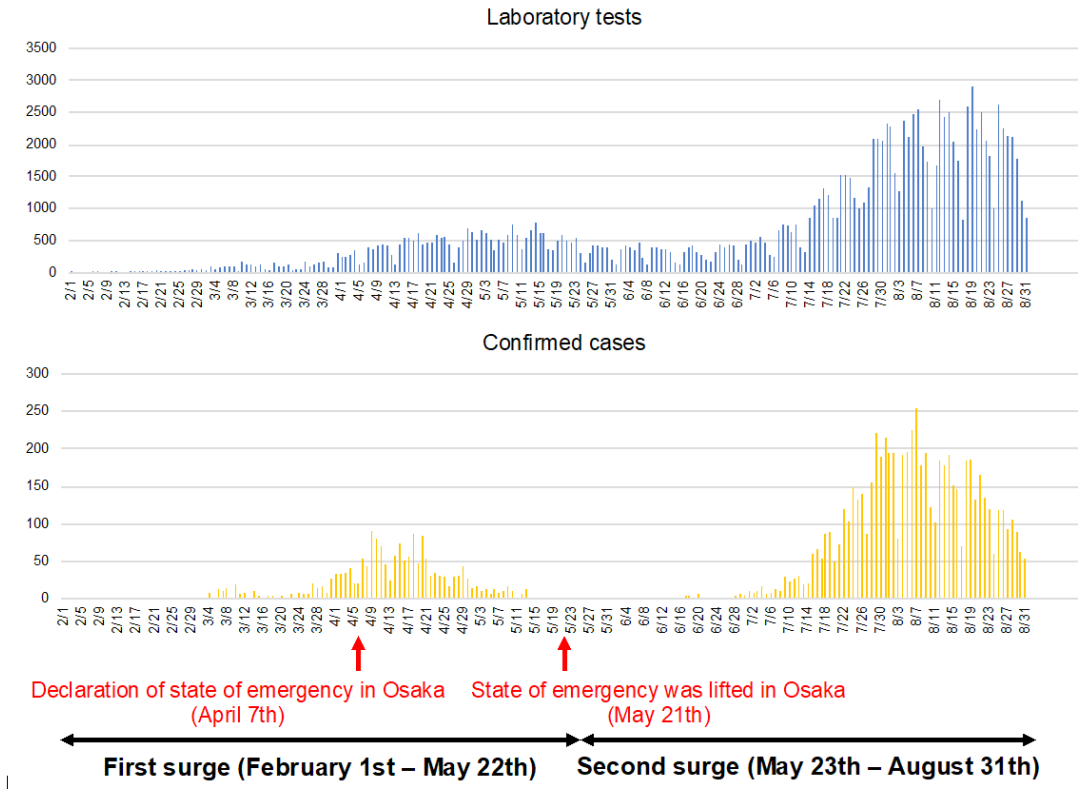

Figure 1. The number of laboratory tests and confirmed coronavirus disease (COVID-19) cases per day in Osaka Prefecture, Japan.

Table 1. Characteristics of the study population according to surge

Variables	First surge February 1 - May 22	Second surge May 23 - August 31	*p* value
	n = 1,780	*n* = 6,761	
Sex			
Female	804 (45.2%)	2,864 (42.4%)	0.08
Male	976 (54.8%)	3,895 (57.6%)	
Unknown	0 (0.0%)	2 (0.0%)	
Age group at the onset date			
0-59	1,292 (72.6%)	5,531 (81.8%)	< 0.001
60-69	161 (9.0%)	419 (6.2%)	
70-79	176 (9.9%)	415 (6.1%)	
80-89	117 (6.6%)	307 (4.5%)	
≥ 90	33 (1.9%)	89 (1.3%)	
Unknown	1 (0.1%)	0 (0.0%)	
City of residence			
Osaka City	737 (41.4%)	3,627 (53.6%)	< 0.001
Other cities	876 (49.2%)	3,075 (45.5%)	
Unknown	167 (9.4%)	59 (0.9%)	
Days to test positive	7 (5-10)	6 (4-8)	< 0.001

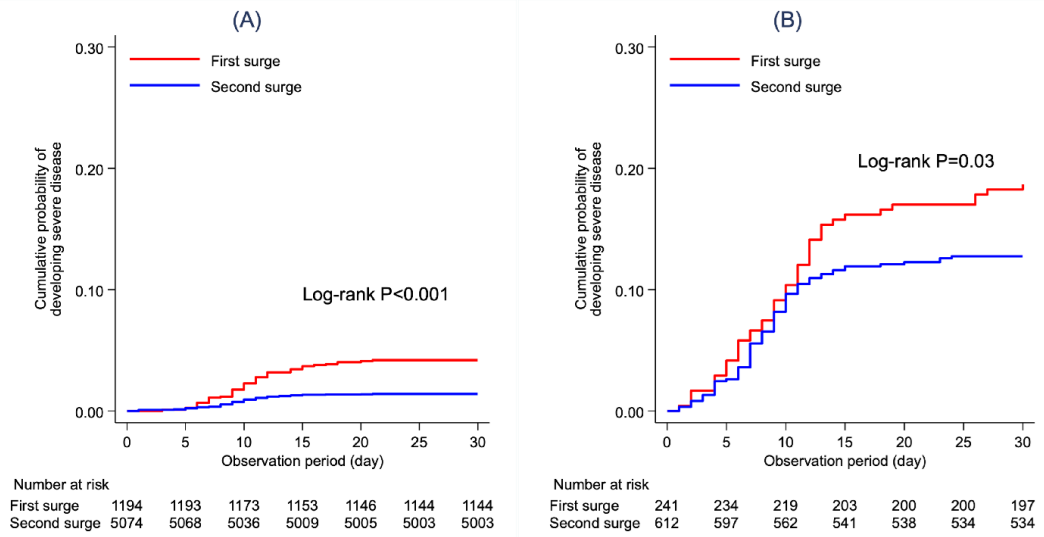

Figure 2. Kaplan-Meier curves for severity among patients aged (A) ≤ 69 years and (B) ≥ 70 years.

Table 2. The results of Cox regression analysis on severity

Variables	Number of patients	Number of events	Adjusted HR (95% CI)
(i) All patients†			
First surge (February 1 - May 22)	1,436	95	*Ref*
Second surge (May 23 - August 31)	5,686	149	0.51 (0.39-0.67)
(ii) Patients aged ≤ 69 years			
First surge (February 1 - May 22)	1,194	50	*Ref*
Second surge (May 23 - August 31)	5,074	71	0.51 (0.35-0.74)
(iii) Patients aged ≥ 70 years			
First surge (February 1 - May 22)	241	45	*Ref*
Second surge (May 23 - August 31)	612	78	0.64 (0.43-0.93)

HR, hazard ratio; CI, confidence interval. †In this model, sex, age group at the onset date (0-59, 60-69, 70-79, 80-89, ≥ 90 years, unknown), city of residence (Osaka City, other cities, unknown), and days to test positive (categorized by quartile) were adjusted.

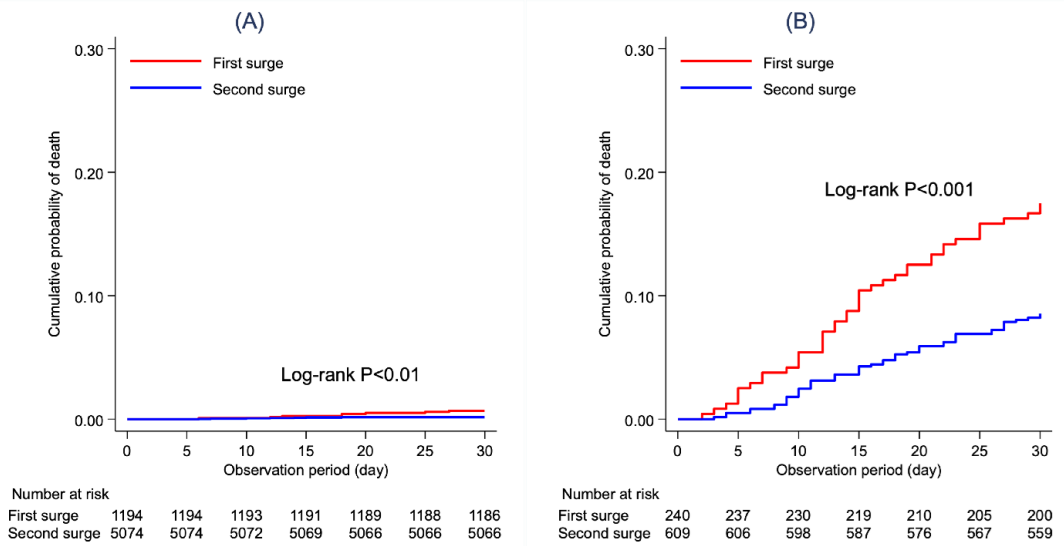

Figure 3. Kaplan-Meier curves for mortality among patients aged (A) ≤ 69 years and (B) ≥ 70 years.

Table 3. The results of Cox regression analysis on mortality

Variables	Number of patients	Number of deaths	Adjusted HR (95% CI)
(i) All patients[†]			
First surge (February 1 - May 22)	1,435	50	*Ref*
Second surge (May 23 - August 31)	5,683	60	0.37 (0.25-0.56)
(ii) Patients aged ≤ 69 years			
First surge (February 1 - May 22)	1,194	8	*Ref*
Second surge (May 23 - August 31)	5,074	8	0.36 (0.13-1.00)
(iii) Patients aged ≥ 70 years			
First surge (February 1 - May 22)	240	42	*Ref*
Second surge (May 23 - August 31)	609	52	0.38 (0.25-0.59)

HR, hazard ratio; CI, confidence interval. [†]In this model, sex, age group at the onset date (0-59, 60-69, 70-79, 80-89, ≥ 90 years, unknown), city of residence (Osaka City, other cities, unknown), and days to test positive (categorized by quartile) were adjusted.

curves for mortality among patients aged ≤ 69 years and ≥ 70 years, respectively. A total of 7,118 patients were included in the analysis of mortality. The log-rank test revealed that mortality was significantly lower in the second surge than in the first surge in each stratum ($p < 0.01$ and $p < 0.001$, for patients aged ≤ 69 years and ≥ 70 years, respectively). Table 3 presents the number of patients, number of events, and adjusted HR with 95% CI by the multivariable Cox proportional-hazards model for mortality. Mortality was lower in the second surge than in the first surge (adjusted HR: 0.37, 95% CI: 0.25-0.56). In the stratified analysis, mortality was lower in the second surge than in the first surge among both patients aged ≤ 69 years and those aged ≥ 70 years (HR: 0.36 and 0.38, for patients aged ≤ 69 years and ≥ 70 years, respectively). Supplementary Table S3 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=11*) describes HRs and 95% CIs for all the variables adjusted in the multivariable Cox regression analysis on mortality. Higher mortality was observed among male than among female (adjusted HR: 1.99, 95% CI: 1.32-3.02). Higher age was significantly associated with higher mortality. Mortality was similar regardless of the city of residence. Lower mortality was observed in the highest and the second highest quartiles of days to test positive compared with the lowest quartile.

Discussion

The present study targeted 8,541 COVID-19 patients between February 1 and August 31, 2020 in Osaka Prefecture, Japan, and compared the characteristics, severity, and mortality of patients between the first and the second surges of infections. In the second surge, the age group at the onset date was younger, the number of days to a positive test was lower, and the severity and mortality were lower.

In this study, both severity and mortality were lower in the second surge than in the first surge, even when we adjusted for available confounding factors. We speculate that this result could be explained in part by the following four factors: *i*) advancement in treatments for

COVID-19; *ii*) behavioral changes and awareness among citizens at higher risk of developing severe disease; *iii*) lower number of clusters at nursing homes; and *iv*) attenuation of SARS-CoV-2.

As of September 30, 2020, there are few drug therapies and no vaccine for COVID-19. However, the treatments for COVID-19 patients have improved over time. Several drug therapies have been approved in Japan. Remdesivir, a nucleotide analog prodrug that inhibits viral RNA polymerases (*10*), was fast-track approved in Japan by the Ministry of Health, Labour, and Welfare on May 7, 2020 (*11*). Dexamethasone, a steroid drug, was also fast-track approved in Japan by the Ministry of Health, Labour, and Welfare in July 2020 (*12*). Clinical trials for other potential drug therapies including Favipiravir, are also in progress (*11*). Intensive care for patients with severe COVID-19, including mechanical ventilation and extracorporeal membrane oxygenation (ECMO) (*11*), has also been improved. Based on the results of clinical studies on COVID-19 patients and improvements in treatments for COVID-19, a guide for the clinical management of patients with COVID-19 for front-line healthcare workers is now available online (*11*). Several scientific societies have also published guidelines for treatment of COVID-19 patients (*13*), and evidence on clinical care of COVID-19 patients has been accumulated. We speculate that improvements in treatments for COVID-19 patients may have resulted in lower severity and mortality in the second surge than in the first surge.

Behavioral changes and awareness over time among Japanese citizens at higher risk of developing severe disease may also have resulted in lower severity and mortality in the second surge. The three Cs, namely closed spaces, crowded places, and close-contact settings (*4*), have been gradually recognized as important for the spread of the COVID-19 pandemic, and new lifestyles termed as the "new normal" lifestyles including maintaining social distancing and using hand sanitizers have gradually become established among Japanese citizens. In particular, a previous study (*14*) reported that such behavioral changes and awareness were more frequently observed among elderly people compared to young people. Elderly people are reported to be at higher risk of developing severe disease and that of death among COVID-19 patients (*6,15-18*). Therefore, we speculate that behavioral changes and awareness among citizens at higher risk of developing severe disease may have led to the lower proportion of these citizens among COVID-19 patients, which may have resulted in lower severity and mortality in the second surge.

Fewer clusters at nursing homes in the second surge than in the first surge would also be associated with lower severity and mortality in the second surge. Previous studies (*19,20*) reported that mortality from COVID-19 was high among residents of nursing homes. Such residents are generally frail (*19*), and they are speculated to be at higher risk for developing severe disease compared with elderly citizens who do not live in nursing homes. We speculate that there were fewer clusters at nursing homes in the second surge based on experiences in the first surge, which would have resulted in lower severity and mortality.

Lower severity and mortality could also be explained in part by the attenuation of SARS-CoV-2. Previous *in vitro* studies on SARS-CoV, which was responsible for the SARS pandemic, showed that deletion in SARS-CoV genome led to lower efficiency of SARS-CoV replication, which could have resulted in milder clinical illness (*21,22*). Such a mutation was also reported in SARS-CoV-2, and was reported to be associated with a milder infection (*23*). Although the effect of mutation on the COVID-19 pandemic is yet to be elucidated, mutation in the SARS-CoV-2 genome may have resulted in lower severity and mortality in the second surge. Further genome and virus studies would be needed to elucidate the attenuation of SARS-CoV-2 in Japan.

In our study, the proportion of elderly patients was lower in the second surge than in the first surge. This result could also be explained in part by two reasons: *i*) behavioral changes and awareness among elderly people who were at higher risk of developing severe disease (*14*); and *ii*) an increased number of laboratory tests for patients who did not present any symptoms in the second surge, especially among younger citizens.

Days to test positive were also significantly lower in the second surge than in the first surge. As we have described in a previous report (*6*), the laboratory testing system for COVID-19 was not widely adopted in Japan in the first surge. Therefore, it is speculated that it took longer for COVID-19 patients to be detected as positive in the first surge. However, by May 2020, the laboratory testing system became more available. Although the maximum number of daily tests was below 1,000 in the first surge, this number was approximately 3,000 in the second surge, as shown in Figure 1. It is speculated that widespread use of laboratory testing systems led to earlier detection of COVID-19 patients. However, fewer days to test positive was not associated with lower severity and mortality, as shown in Supplementary Tables S2 and S3 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=11*). In addition, higher mortality was observed among patients in the fewest category (Q1) of days to test positive compared with the highest (Q4) and second highest (Q3) categories. The proportion of patients aged ≥ 70 years was highest in Q1 (Supplementary Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=11*). In Osaka Prefecture, when elderly facility residents and clusters among elderly patients were suspected to be infected with COVID-19, laboratory tests for such residents were conducted as soon as possible. We speculate that such residents, who were at higher risk of developing severe disease, underwent laboratory tests

rapidly; even though, some proportion of these residents developed severe disease or died subsequently.

Previous studies have compared the characteristics of COVID-19 patients between the first and the second surges. A previous study conducted in Texas in the United States (*5*) compared the characteristics and outcomes of COVID-19 patients between surge 1 (March 13, 2020 to May 15, 2020) and surge 2 (May 16, 2020 to July 7, 2020), and found that patients in surge 2 were younger and had lower in-hospital mortality in surge 2 (*5*), both of which were consistent with our findings. A previous study (*24*) in Wuhan categorized patients into three groups: group A (January 21 to January 25), group B (January 26 to January 31), and group C (February 1 to February 10), and found that all-causes of mortality significantly decreased over time (*24*); this was also consistent with our findings. A recent study in Japan (*25*) compared characteristics and severity between the first and second COVID-19 waves in Japan, and concluded that the proportion of severe cases on admission was lower in the second wave. Further studies are needed to examine changes in the characteristics of COVID-19 patients over time. Especially, from November 2020, since Japan is in the middle of the third surge of infections (*4*). In Osaka Prefecture, as of December 5, 2020, the daily number of newly diagnosed COVID-19 patients was the highest ever on November 22, 2020 (490 cases per day) (*7*). Future studies will be needed to compare severity and mortality between the first, second, and third surge of infections.

There were several limitations to the present study. First, we did not have information on patient age (not age group) at the onset. We could not adjust for age in the multivariable analysis. Instead, the age group at the onset date was adjusted, which could have resulted in residual confounding. Second, we could not compare causes of death among COVID-19 patients between the first and the second surges, because this information was not publicly available (*6*). Third, we could not include patients with missing information on the onset date/date when the patient's condition became severe/date of death in the survival analyses. Finally, information on patient characteristics such as medical history, comorbidities, in-hospital treatments, and health status was not publicly available, and we could not adjust for these factors in the multivariable analysis (*6*).

In conclusion, in Osaka Prefecture, Japan, severity and mortality were significantly lower in the second surge than in the first surge, among COVID-19 patients. It was also concluded that the number of days to test positive was lower in the second surge than in the first surge. Further studies are needed to analyze the severity and mortality in COVID-19 patients. Although the results of this study cannot explain why severity and mortality was lower in the second surge, greater advancement in treatments for COVID-19, behavioral changes and awareness among citizens, and effective measures to prevent clusters at nursing homes are needed for disease control. In addition, intensive and careful treatments are needed for elderly and male patients, who are at higher risk.

Acknowledgements

Takeuchi T was supported by the Osaka University Medical Doctor Scientist Training Program. We thank our colleagues from Osaka University Center of Medical Data Science and Advanced Clinical Epidemiology Investigator's Research Project for providing insight and expertise for our research.

Funding: None.

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

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Received October 28, 2020; Revised December 10, 2020; Accepted December 15, 2020.

Released online in J-STAGE as advance publication December 30, 2020.

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Global Health & Medicine. 2021; 3(2):90-94.

ORIGINAL ARTICLE

DOI: 10.35772/ghm.2021.01022

Consistency of the results of rapid serological tests for SARS-CoV-2 among healthcare workers in a large national hospital in Tokyo, Japan

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Abstract: We assessed the consistency of seropositive results of three rapid immunoassays (Kits A, B, and C) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) compared to highly accurate serological tests (Abbott and Roche) among healthcare workers in a hospital in Tokyo. The seroprevalence of SARS-CoV-2 immunoglobulin G was 0.41%, 2.36%, and 0.08% using Kits A, B, and C, respectively. Of the 51 samples that were seropositive on any rapid test, all were seronegative on both the Abbott and the Roche assays. Given that the seroprevalence of SARS-CoV-2 immunoglobulin G varied widely according to the choice of rapid test and the rapid test results were inconsistent with the results of highly accurate tests, the diagnostic accuracy of rapid serological tests for SARS-CoV-2 should be assessed before introducing these tests for point-of-care testing or surveillance.

***Keywords*:** COVID-19, serological tests, SARS-CoV-2, seroprevalence, immunoassay

Introduction

Accurate and rapid testing is required for the diagnosis of coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has spread rapidly worldwide since December 2019. There are two main categories of diagnostic tests for COVID-19: molecular tests that detect SARS-CoV-2 ribonucleic acid (RNA) and serological tests that detect anti-SARS-CoV-2 immunoglobulins. Reverse transcriptase-polymerase chain reaction (RT-PCR), a molecular test, is widely used as the reference standard for the diagnosis of SARS-CoV-2 infection, while serological tests have generated an interest as an alternative or complement to RT-PCR for diagnosing acute infection.

Several serological tests are available, including laboratory-based (*e.g.*, enzyme-linked immunosorbent assay [ELISA] and chemiluminescent immunoassay [CLIA]) and rapid diagnostic tests (*e.g.*, lateral flow immunoassay [LFIA]). In particular, LFIA-based tests are inexpensive, rapid, and easy to implement at point-of-care. This has stimulated the development and marketing of LFIA commercial kits (*1*). However, the pace of development has exceeded that of rigorous evaluation, and critical uncertainty about their accuracy remains (*1*). A systematic review and meta-analysis of the diagnostic accuracy of serological tests for SARS-CoV-2 found evidence that sensitivity and specificity of tests that use the LFIA method may be lower than those of tests that use ELISA or CLIA methods (*2*).

To date, few studies have examined the consistency of seropositive results from multiple rapid tests. Studies conducted in the United States (*3*), United Kingdom (*4*), and Denmark (*5*) have found that commercial LFIA kits showed varying levels of diagnostic accuracy for SARS-CoV-2. In Japan, however, no studies have assessed the level of agreement of multiple rapid serological tests for detecting SARS-CoV-2 infection. Several studies have found a seroprevalence ranging from 0.03-0.88% on laboratory-based tests and 0.2-8.53% on rapid tests in the general Japanese population,

outpatients, and healthcare workers (HCWs) (*6-9*). The seroprevalence estimate in different studies has varied widely and appears to be higher with rapid tests than with laboratory-based tests. Differences in the diagnostic accuracy between tests and the repeatability within tests, makes it difficult to compare seroprevalence estimates across studies.

In the present study, we investigated whether the results of three types of rapid LFIA were consistent with those of two types of highly accurate laboratory-based tests for estimating the seroprevalence of SARS-CoV-2 infection among healthcare workers in a large hospital in Tokyo, Japan.

Materials and Methods

Study design and participants

This cross-sectional study was conducted in July 2020 among workers at the National Center for Global Health and Medicine (NCGM), a leading institute working on combating COVID-19 in Japan. The survey targeted mainly those engaged in COVID-19-related work or who worked in a department with a high risk of exposure to SARS-CoV-2 infection. The seroprevalence of SARS-CoV-2 measured using the two laboratory-based tests among the study participants was reported elsewhere (*10*).

The study protocol was approved by the Ethics Committee of the National Center for Global Health and Medicine, Japan. Written informed consent was obtained from all participants.

Serological tests

We used three LFIA rapid tests from different manufacturers (Kits A, B, and C), performed according to each manufacturer's instructions, to determine whether samples were anti-SARS-CoV-2 immunoglobulin M (IgM) and/or immunoglobulin G (IgG) positive. Serum separated from a blood sample of a brachial vein was used for the SARS-CoV-2 antibody tests with the Abbott and Roche tests, and Kits A and B, while a finger-prick whole blood sample was used for tests with Kit C. Kits A and B were conducted by medical laboratory technicians. Samples that were positive were retested, and the result of the repeat test was adopted. Kit C was performed by trained staff, and used a finger-prick blood sample, so each sample was tested only once. The result was checked by at least two trained staff. Kit A had a reported sensitivity of 87.9% and 97.2%, and specificity of 100% and 100% for measuring IgM and IgG, respectively. Kits B and C had a reported sensitivity of 87.3% and 90.4%, respectively, and 100% specificity. Kits B and C did not differentiate between IgM or IgG.

The Abbott test was run on the Abbott Architect analyzer, using the SARS-CoV-2 IgG assay, which is based on the chemiluminescent microparticle immunoassay method for the qualitative detection of IgG in human serum or plasma against the SARS-CoV-2 nucleoprotein. A value of 1.40 or higher was considered positive. This assay was reported to have 99.9% specificity and 100% sensitivity for detecting the IgG antibody 17 days after symptoms onset (*11*).

The Roche test was run on the Roche cobas® e602 analyzer, using the Elecsys® Anti-SARS-CoV-2, based on the electrochemiluminescence immunoassay for the *in vitro* qualitative detection of total antibodies (including IgG) to the SARS-CoV-2 nucleoprotein. A value of 1.00 or higher was considered positive. This assay was reported to have 99.8% specificity and 100% sensitivity 14 days after symptoms onset.

We performed post hoc testing of samples with positive results on the Abbott or Roche tests, Kit B, two or more kits, and samples that were close to the index threshold on the Abbott or Roche tests using EUROIMMUN anti-SARS-CoV-2 IgG ELISA. The EUROIMMUN test methods are described in the Supplementary Materials (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=18*).

Statistical analysis

We calculated the seroprevalence of SARS-CoV-2 antibody and its 95% confidence interval for each test. We drew scatterplots to display the index values of the Abbott and Roche tests of the samples that were positive on any of the tests. The data were analyzed using Stata version 16 (Stata Corp., College Station, TX, USA).

Results

Participant characteristics

Of 1,579 workers recruited, 1,228 agreed to participate and completed the questionnaire, and at least one serological test was conducted. The mean (± standard deviation) age of participants was 36.1 ± 11.0 years, and 29% were men. The primary job categories represented were nurses (49%), physicians (19%), and allied healthcare professionals (14%). Only one of the 91 participants who self-reported that they had previously received a PCR test for SARS-CoV-2 (timing unknown) tested positive on the PCR test.

Seroprevalence according to each rapid test

Table 1 shows the seroprevalence of SARS-CoV-2 antibodies using each serological test. All participants received tests using Kits A and B (n = 1,228), while 1,197 had test results available for the Kit C test because ten refused to provide a finger-prick blood sample

Table 1. Results of multiple serological tests for SARS-CoV-2 among workers in a large national hospital in Tokyo, Japan

	Abbott	Roche	Kit A		Kit B		Kit C	
			IgG	IgM	IgG	IgM	IgG	IgM
Subjects	*n* = 1,228	*n* = 1,228	*n* = 1,228	*n* = 1,228	*n* = 1,228	*n* = 1,228	*n* = 1,197	*n* = 1,197
Seropositive, *n*	1	1	29	25	5	8	1	0
Seroprevalence, %	0.08	0.08	2.36	2.04	0.41	0.65	0.08	0
(95% CI)	(0.02-0.45)	(0.02-0.45)	(2.59-3.37)	(1.32-3.00)	(0.13-0.95)	(0.28-1.28)	(0.00-0.46)	(0.00-0.00)
Pattern A (*n* = 1)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Pattern B (*n* = 1)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Pattern C (*n* = 2)	(-)	(-)	(+)	(+)	(-)	(+)	(-)	(-)
Pattern D (*n* = 12)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(-)
Pattern E (*n* = 1)	(-)	(-)	(+)	(-)	(-)	(-)	(+)	(-)
Pattern F (*n* = 14)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)
Pattern G (*n* = 11)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
Pattern H (*n* = 5)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)
Pattern I (*n* = 6)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)

CI, confidence interval. (+): positive; (-): negative. Abbott: chemiluminescent microparticle immunoassay (CMIA). Roche: electrochemiluminescence immunoassay (ECLIA). Kit A, B, and C: lateral flow immunoassay (LFIA).

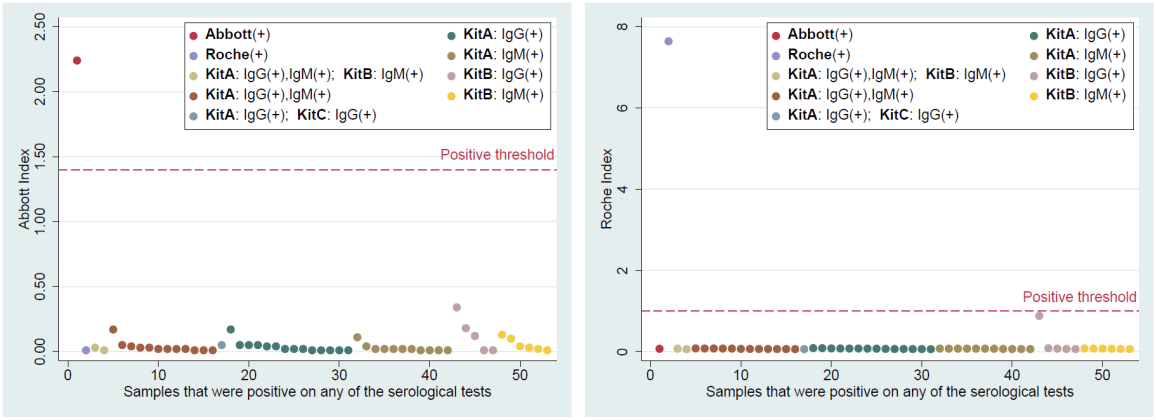

Figure 1. Individual index values of the Abbott (left panel) or Roche (right panel) tests in samples that were tested seropositive on any serological test. The Y-axis shows the Abbott or Roche index values. The X-axis shows the results of 53 individuals who were positive on any of the serological tests. (+): positive; (-): negative. The positive threshold value of the Abbott test is 1.40. The positive threshold value of the Roche test is 1.00.

for testing, and 21 had indeterminate test results. The seroprevalence of IgG on Kits A, B, and C was 2.36% (95% confidence interval [CI]: 2.59-3.37%), 0.41% (95% CI: 0.13-0.95), and 0.08% (95% CI: 0.00-0.46%), respectively, and the seroprevalence of IgM was 2.04% (1.32-3.00%), 0.65% (0.28-1.28%), and 0% (0.00-0.00%), respectively.

Consistency of serological test results

Table 1 shows the consistency of results across the serological tests for participants having at least one positive result. Few participants showed an agreement of seropositive results across the rapid tests: two were IgM seropositive on Kits A and B, and one was IgG seropositive on Kits A and C. One participant who self-reported having tested positive on the PCR test for SARS-CoV-2 was negative on all serological tests.

The two participants who were positive on either the Abbott or the Roche test were negative on all three rapid tests. Figure 1 shows the Abbott and Roche index for each positive sample. None of the samples with positive results on any of the rapid tests had an Abbott index close to the positive threshold (1.40). One individual who was positive on Kit B (IgG) had a Roche index of 0.879, which is close to the positive threshold (1.00).

Post hoc testing using the EUROIMMUN test

Samples that were seropositive on the Abbott test, Roche test, Kit B (IgG), or two kits, and those who were close to the threshold of the index of the Abbott or the Roche test were all negative on post hoc testing using the EUROIMMUN test (Supplementary Figure S1, *https://*

www.globalhealthmedicine.com/site/supplementaldata.html?ID=18).

Discussion

In the present study, we assessed the consistency of the seropositivity of three types of rapid tests for SARS-CoV-2 antibodies compared to the results of the Abbott and Roche tests among HCWs in a large hospital designated for the care of COVID-19 patients in Tokyo, Japan. None of the 51 samples that were seropositive on any of the rapid tests were positive on either the Abbott or the Roche tests, and only three cases had consistently seropositive results using two different rapid tests.

In Japan, studies using a rapid SARS-CoV-2 antibody test have found varying seroprevalence (1.79-9.1%) among HCWs (*8,9,12*). SoftBank Corp conducted a survey using Kit B among 5,850 HCWs at multiple medical institutions across Japan in May and June 2020 and found a seroprevalence of 1.79% (*9*). Another study measured the antibody among 55 HCWs of two clinics in Tokyo using a different type of kit in April 2020 and found a seroprevalence of 9.1% (*8*). The difference in seroprevalence found in previous studies could reflect the background of each HCW and timing of measurement (*13*). Nevertheless, given the marked difference in seropositivity according to each of the three rapid tests in the present population, the use of different rapid test kits may be one of the sources of variation.

The growing body of literature on the accuracy of antibody tests for SARS-CoV-2 shows that the sensitivity and specificity vary widely between rapid tests (*3-5*). A study that compared the accuracy of ten different rapid tests (IgG) found sensitivities and specificities ranging from 66.7-90.9% and 91.6-100%, respectively (*3*). In the present study, all samples that were seropositive on any rapid test were negative on the highly specific Abbott and Roche tests, suggesting that all the positive results of the rapid tests were false-positive results. If the prevalence of the outcome is low, a test with a low specificity will produce many false positives and overestimate the positive rate. For example, if 1% of the population is infected with a virus, a test with sensitivity and specificity of 100% and 92% would lead to a prevalence of 9% (89% of all test positives are false positives). The considerably higher seroprevalence (IgG) obtained by using Kit A (2.36%) than those obtained by using Kits B: (0.41%) and C (0.08%), suggests that Kit A has a relatively low specificity. In populations with a very low prevalence of SARS-CoV-2 infection, such as current Japan, it is crucial to adopt a test with a high specificity to estimate seroprevalence accurately.

Rapid test kits may tend to misidentify other viral antibodies as SARS-CoV-2 positive (*14*). For example, of seven stored serum samples of patients with human common cold coronavirus pneumonia admitted up until January 2019, four were identified to be SARS-CoV-2 positive using a rapid test (*15*). In a systematic review and meta-analysis on the diagnostic accuracy of SARS-CoV-2 serological tests (*2*), rapid tests tended to show a higher frequency of false-positive results (*i.e.*, lower specificity) than laboratory tests when blood samples of patients without a history of COVID-19 but with a common cold or another viral infection were used as negative controls. Given these data, it is necessary to evaluate the specificity using blood samples from patients infected with a virus similar to SARS-CoV-2.

A key limitation of the present study is the lack of a "gold standard" for SARS-CoV-2 antibody testing. We confirmed the presence of the antibodies using reliable laboratory-based tests (Abbott and Roche), but these are not perfect measures; for example, Perkmann *et al.* (*16*) reported that the sensitivity and specificity were 84.6% and 99.2% for the Abbott test, and 89.2% and 99.7% for the Roche test. In fact, our post hoc analysis revealed that the two participants with seropositive Abbott or Roche tests were negative using the EUROIMMUN quantitative antibody test, suggesting that both results were false-positive results. This result is as expected in a setting of very low seroprevalence, even using highly specific tests, such as the Abbott and Roche tests.

In conclusion, the estimated seroprevalence of SARS-CoV-2 infection varied widely across the three rapid tests, and samples that were seropositive on any rapid test were negative on the highly accurate Abbott and Roche tests. The accuracy of rapid tests should be carefully evaluated before introducing these assays as point-of-care tests or for surveillance.

Acknowledgements

We thank the researchers and research assistants in the Department of Epidemiology and Prevention, National Center for Global Health and Medicine, for scientific advice and administrative support on the study's conduct. The reagents/kits for the SARS-CoV-2 antibody testing used in this study were provided by Abbott Japan LLC, Roche Diagnostics K.K., Loki Consulting Co, Ltd., and SoftBank Corp.

Funding: This work was supported by NCGM COVID-19 Gift Fund from the National Center for Global Health and Medicine and the Japan Health Research Promotion Bureau Research Fund (2020-B-09).

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

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Received February 18, 2021; Revised March 23, 2021; Accepted March 25, 2021.

Released online in J-STAGE as advance publication April 2, 2021.

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Global Health & Medicine. 2021; 3(2):95-101.
ORIGINAL ARTICLE
DOI: 10.35772/ghm.2021.01025

Elevated high-sensitivity troponin is associated with subclinical cardiac dysfunction in patients recovered from coronavirus disease 2019

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Abstract: The aim of this study is to investigate myocardial damage in recovering coronavirus disease 2019 (COVID-19) patients with high-sensitivity troponin levels (hsTnT) and echocardiography. In this single-center cohort study, 215 COVID-19 recovered patients were recruited from all over Japan between April and September 2020. Demographic characteristics, hsTnT levels, and echocardiography data were collected for 209 patients, after excluding those without serum samples or good-quality echocardiographic images. The mean (± standard deviation) age was 44 (± 12) years (range: 36-55 years), and 50.7% of the patients were males. The median time interval (interquartile range) from COVID-19 onset to post-recovery examination was 56 days (34-96 days). Seventy-four recovered patients (35.4%) had hsTnT less than detection sensitivity (< 3 pg/mL) and 135 recovered patients (64.6%) had hsTnT ≥ 3 pg/mL. Ejection fraction was more than 50% in all cases. Left ventricular global longitudinal strain (LVGLS) and right ventricular free-wall longitudinal strain (RVFWLS) were reduced in 62 (29.7%) and 8 patients (3.8%), respectively. They were significantly associated with elevated hsTnT levels. In cases with hsTnT above 5 pg/mL, the LVGLS was greatly reduced to 19.0 ± 2.2% ($p < 0.001$). Multivariate linear regression analysis showed that elevated hsTnT level was an independent predictor of reduced LVGLS (standardized $\beta = -0.34$; $p < 0.001$). In recovered COVID-19 patients, even a slight increase in hsTnT above detection sensitivity was associated with decreased LVGLS. hsTnT and echocardiography may be useful tools to detect myocardial injury in recovered COVID-19 patients.

***Keywords*:** COVID-19, myocardial injury, high-sensitivity troponin levels, left ventricular global longitudinal strain, COVID-19 sequelae

Introduction

Coronavirus disease 2019 (COVID-19) is a threat in terms of prevalence and mortality worldwide (*1*). Myocardial damage in patients with COVID-19 infection has been reported (*2,3*). Several case reports and small series have suggested that elevated troponin in COVID-19 patients has a significant impact on worsening cardiovascular disease and death (*4-6*). Recently, a study reported an association between high-sensitivity troponin T (hsTnT) levels slightly elevated above the detection sensitivity and myocardial damage on magnetic resonance imaging (MRI) in recovered COVID-19 (*7*). In addition, cardiac MRI abnormalities were detected in mild cases of COVID-19 among young athletes (*8*). It was also reported that strain analysis by echocardiography performed during hospitalization was associated with death (*9-11*). In particular, myocardial strain analysis by echocardiography has shown that a reduction in left and right ventricular longitudinal strain is an independent prognostic factor (*12,13*). Therefore, it is assumed that abnormalities in cardiac functions seen on an echocardiogram may be prolonged even after the recovery of the infection. In addition, there are no studies on the detailed assessment of cardiac functions by echocardiography during the recovery period, including studies on patients with mild or poor symptomatic disease and no history of oxygen inhalation.

The aim of this study was to evaluate the presence of myocardial damage using serological myocardial damage markers and echocardiography in patients who had recently recovered from COVID-19 disease.

Patients and Methods

Study design and population

Patients who participated in COVIPLA, a study on convalescent plasma therapy in Japan between April and September 2020, were enrolled in this study.

Patients ranged in age from 20 to 70 years, and all participants had negative results on swab tests and underwent blood tests and echocardiography at least 3 weeks after onset of infection. All examinations in the COVID-19 recovery period were performed in the National Center for Global Health and Medicine. In addition, patients weighing more than 45 kg among males and more than 40 kg among females were enrolled.

This study conforms to the guidelines laid down by the Declaration of Helsinki, and the protocol was approved by the hospital ethics committee (NCGM-G-003559-01). An opportunity for eligible patients to refuse to participate in this study was provided by opting out format.

Measurement of hsTnT

Measurement of hsTnT was performed using a commercially standardized sample kit (Roche Diagnostics, Tokyo, Japan) on the serum samples stored at -80°C obtained from the COVIPLA registry.

Echocardiographic data analysis

Echocardiography was performed on the same day as that of blood sampling. Comprehensive echocardiographic examination was performed using commercially available ultrasound machines (Artida; Toshiba Medical Systems, Tokyo, Japan) by trained and registered echocardiographers who were blinded to the clinical information of the patients.

Ejection fraction (EF) and left ventricular (LV) end-diastolic and end-systolic volumes were calculated using the disk summation method. Right ventricular (RV) end-diastolic area and RV fractional area change (RVFAC) were measured from the apical 4-chamber view to approximate RV size and systolic function, respectively. Tricuspid annular plane systolic excursion (TAPSE) was measured from the apical four-chamber view with the M-mode cursor through the lateral tricuspid annulus. Cardiac function analysis was performed by a cardiology specialist and a clinical technician specializing in echocardiography. In speckle tracking analysis, LV global longitudinal strain (LVGLS), circumferential strains, and RV longitudinal strain (RVLS) were measured using Image Arena (TOMTEC Imaging Systems, Germany). LVGLS was calculated from the averages of the 4-chamber, 3-chamber, and 2-chamber views, and RVLS was calculated from the 4-chamber view to obtain RV 4-chamber strain including the ventricular septum (RV4CSL) and right ventricular free-wall longitudinal strain (RVFWLS) (*14*).

In this study, strain values were expressed in absolute values, and larger absolute values indicated better cardiac ventricular function. Myocardial strain analysis was performed by two independent, blinded observers. Ventricular dimensions, volume procedures, and reference values for abnormalities were based on the guidelines of the American Society of Echocardiography and the European Society of Cardiovascular Imaging (*15*). As a control group for right and left ventricular myocardial strain, age, sex and hypertension matched patients (n = 30) with no history of cardiovascular disease were referred.

Statistical analysis

Continuous measures were shown as mean ± standard deviation, and if the variables were normally distributed, *t*-test and ANOVA were used to compare differences between two and three groups, respectively. If the variables were not normally distributed, they were expressed as median values (25th-75th percentile) and compared between two groups using the Mann-Whitney *U* test and between three groups using the Kruskal-Wallis test. Comparisons among the three groups were performed using the Tukey-Kramer post hoc test or the Dunn correction for post hoc analyses, according to the distribution of the test.

Categorical variables were presented as numbers and percentages and compared using the chi-squared test or the Kruskal-Wallis test. Correlations of continuous variables were tested with the Pearson's correlation coefficient if the data were normally distributed. A two-sided p-value < 0.05 was considered statistically significant.

In myocardial strain analysis, intra-observer variability by the same observer at two different time points for 15 patients was also analyzed. The results were analyzed using Pearson correlation analysis and the Bland-Altman method.

Multiple linear regression analysis of the association between echocardiographic strain measures and hsTnT levels, including age, sex, and risk of cardiovascular disease risk as independent variables, was performed. Standardized partial regression coefficients (β) were used to compare the effect on the dependent variable, and 95% confidence intervals were determined.

All statistical analyses were performed using IBM SPSS statistical software, version 24 (IBM, Illinois, USA).

Results

Patient characteristics

Two hundred and fifteen patients from the COVIPLA registry who underwent echocardiography and blood tests were enrolled between April and September 2020. We finally included 209 patients after excluding cases in which hsTnT levels could not be measured due to lack of serum samples (n = 5) and in which good-quality echocardiographic images were not available (n = 1). The mean age (± standard deviation) was 44 ± 12 years (range: 36-55 years). The proportion of males was 50.7%. Of the 41 patients (19.6%) with a history of oxygen inhalation, 4 (1.9%) had a history of intubation and 3 (1.4%) had a history of extracorporeal membrane oxygenation.

Elevation of hsTnT

The hsTnT levels were less than the detection sensitivity (< 3 pg/mL) in 74 recovered patients (35.4%) and were ≥ 3 pg/mL in the remaining 135 recovered patients (64.6%). In this study, we compared hsTnT levels between two groups: one with levels less than detection sensitivity (< 3 pg/mL) and the other with levels ≥ 3 pg/mL. The baseline characteristics of patients in these two groups are summarized in Table 1. Comparisons between the groups showed significant differences in age, sex, body mass index (BMI), hypertension, diabetes mellitus, dyslipidemia, oxygen demand on admission, and hemoglobin levels.

Echocardiographic parameters based on hsTnT levels

The LVEF was more than 50% in all cases. According to the guidelines for echocardiographic abnormalities (*14*), LVGLS was reduced (< 20%) in 62 patients (29.7%), TAPSE was < 17 mm in 16 patients (7.7%), and RVFWLS was < 20% in 8 patients (3.8%). A significant difference in the reduction of LVGLS was observed between patients with hsTnT levels below the detection sensitivity (hsTnT < 3) and those with levels ≥ 3 ($p < 0.001$) (Table 1). Table 1 shows the comparison of the mean values of different echocardiographic parameters between the two groups. There were significant differences between the groups in terms of LV end-diastolic and -systolic volumes, LV mass index, LVGLS, and LV diastolic functions (E/A (E: early diastolic trans-mitral flow velocity, A: late diastolic trans-mitral flow velocity) and E/e' (e': early diastolic mitral annular velocity, DT deceleration time)). Right atrial (RA) and ventricular volumes (RA area, RV end-diastolic area) and RV systolic functions (TAPSE, RVLS) were statistically different.

Figure 1 shows a comparison by one-way ANOVA and post hoc analysis of control and hsTnT levels. LVEF was not significantly different among the three groups at the hsTnT level (64.9 ± 4.6%, 65.8 ± 4.3%, and 66 ± 4.9%; $p = 0.36$). The mean value of LVGLS in control group was 22.7 ± 4.1%. There was a significant

Table 1. Baseline characteristics according to hsTnT Levels

Variables	Overall, n = 209	hsTnT < 3, n = 74	hsTnT ≥ 3, n = 135	p-value
Age, y	45 ± 12	38 ± 9	49 ± 12	< 0.001
Males	106 (50.7%)	13 (17.6%)	93 (68.9%)	< 0.001
BMI, kg/m^2	24.3 ± 3.7	22.4 ± 2.0	25.6 ± 4.2	< 0.001
HR, beats/min	65 ± 10	63 ± 9	65 ± 10	0.10
Comorbidities				
Hypertension	33 (15.8%)	3 (4.1%)	30 (22.2%)	< 0.001
Diabetes	16 (7.7%)	0 (0.0%)	16 (11.9%)	0.001
Dyslipidemia	26 (12.4%)	2 (2.7%)	24 (17.8%)	0.001
Smoking	47 (22.5%)	15 (20.3%)	32 (23.7%)	0.63
COPD	1 (0.5%)	0 (0.0%)	1 (0.7%)	1.00
CAD	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Arrhythmia	4 (1.9%)	0 (0.0%)	4 (3.0%)	0.30
Oxygen inhalation	41 (19.6%)	4 (5.4%)	37 (27.4%)	< 0.001
Intubation	5 (2.4%)	1 (1.4%)	4 (3.0%)	0.66
ECMO	0 (0.0%)	0 (0.0%)	2 (1.5%)	0.54
Laboratory				
WBC, × 10^3/μL	5,649 ± 1,434	5,663 ± 1,503	5,591 ± 1,401	0.73
Hemoglobin, g/dL	13.8 ± 1.4	13.1 ± 1.1	14.1 ± 1.4	< 0.001
Creatinine, mol/L	0.71 ± 0.15	0.65 ± 0.1	0.74 ± 0.2	0.03
eGFR, ml/min/1.73 m^2	88.1 ± 11.1	89.2 ± 9.6	87.4 ±12.1	0.27
Echocardiography				
LVEF < 53%	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
LVGLS < 20%	62 (29.7%)	3 (4.1%)	59 (43.7%)	< 0.001
RVFAC < 35%	17 (8.1%)	3 (4.1%)	14 (10.4%)	0.18
TAPSE < 17 mm	16 (7.7%)	3 (4.1%)	13 (9.6%)	0.27
RVFWLS < 20%	8 (3.8%)	0 (0.0%)	8 (6.0%)	0.05

Results are expressed as mean ± standard deviation or number (%). BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; eGFR, estimated glomerular filtration rate; HR, heart rate; hsTnT, high-sensitivity troponin T; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; RVFAC, right ventricular fractional area change; RVFWLS, right ventricular free-wall strain; TAPSE, tricuspid annular plane systolic excursion; WBC, white blood cell count.

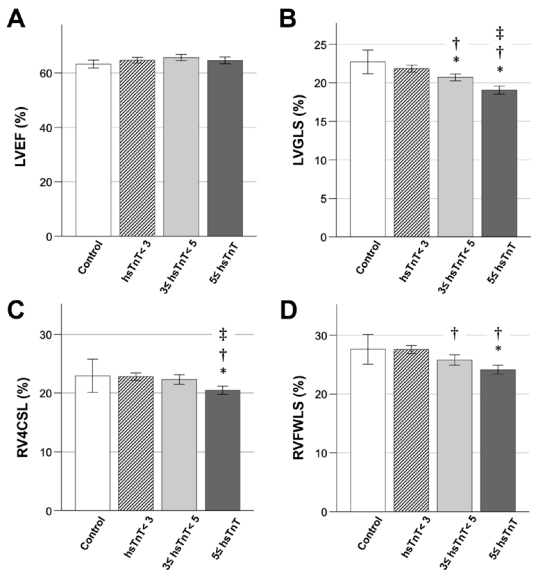

Figure 1. Comparison of cardiac ventricular functions at different hsTnT levels in recovered COVID-19 patients. (A) Significant differences were not detected in LVEF of hsTnT among the four groups. **(B, C,** and **D)** Significant differences in LVGLS, RV4CSL, and RVFWLS between the two groups above hsTnT detection sensitivity ($3 \leq$ hsTnT). $^{*}p < 0.05$ compared with control. $^{\dagger}p < 0.05$ compared with hsTnT < 3. $^{\ddagger}p < 0.05$ compared with $3 \leq$ hsTnT < 5. hsTnT, high-sensitivity troponin; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; RV4CSL, right ventricular 4-chamber strain including the ventricular septum; RVFWLS, right ventricular free-wall longitudinal strain.

difference among the three groups at the hsTnT level (21.8 ± 1.9%, 20.7 ± 1.8%, and 19.0 ± 2.2%; $p < 0.001$). Furthermore, post hoc analysis in the $3 \leq$ hsTnT < 5 and $5 \leq$ hsTnT groups showed a significant difference in LVGLS. RV4CSL and RVFWSL were significantly different among the three hsTnT groups. Significant differences were also detected in RV4CSL (22.4 ± 3.0% and 20.5 ± 2.8%; $p = 0.006$) and RVFWLS (26.0 ± 3.5% and 24.1 ± 3.1%; $p = 0.001$) when the $3 \leq$ hsTnT < 5 group and 5 < hsTnT group were compared. Only groups above the detection sensitivity of hsTnT (> 3 pg/mL), and the parameters that divided these into two groups by a median of 5 pg/mL of hsTnT are shown in Supplementary Table S1(*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=19*). There were significant differences in LVGLS, LV volume, myocardial thickness, RVLS (RV4CSL and RVFWLS), and RA area between the two groups above hsTnT detection sensitivity. A comparison of hsTnT with left and right ventricular function showed a stronger correlation of hsTnT with LVGLS ($r = -0.56$, $p < 0.001$) than with RVFWSL ($r = -0.34$, $p < 0.001$) or EF ($r = -0.02$, $p = 0.74$) (Figure 2).

Multiple linear regression analysis in LVGLS and RVLS

Multiple regression analysis was performed to examine

Table 2. Multivariate linear regression analysis as a predictor of LVGLS and RVFWLS

Variables	LVGLS			RVFWLS		
	β	95% CI	*p*-value	β	95% CI	*p*-value
Age	-0.14	-0.05 to -0.001	0.04	-0.23	-0.11 to -0.02	0.005
Sex (male)	-0.26	-1.78 to -0.62	< 0.001	-0.15	-2.0 to -0.06	0.04
BMI	-0.06	-0.12 to 0.04	0.33	-0.19	-0.3 to -0.05	0.008
Hypertension	-0.23	-0.89 to 0.61	0.71	0.07	-0.67 to 1.9	0.34
Diabetes	-0.06	-1.5 to 0.49	0.31	-0.09	-2.5 to 0.96	0.23
Oxygen inhalation	0.04	-0.46 to 0.88	0.54	-0.02	-1.3 to 0.97	0.76
eGFR	-0.11	-0.04 to 0.0	0.05	-0.12	-0.08 to 0.003	0.04
hsTnT	-0.34	-0.30 to -0.12	< 0.001	-0.14	-0.28 to 0.02	0.08

β, standardizedβ; BMI, body mass index; CI, confidence intervals; eGFR, estimated glomerular filtration rate; hsTnT, high-sensitivity troponin T; LVGLS, left ventricular global longitudinal strain; RVFWLS, right ventricular free-wall strain.

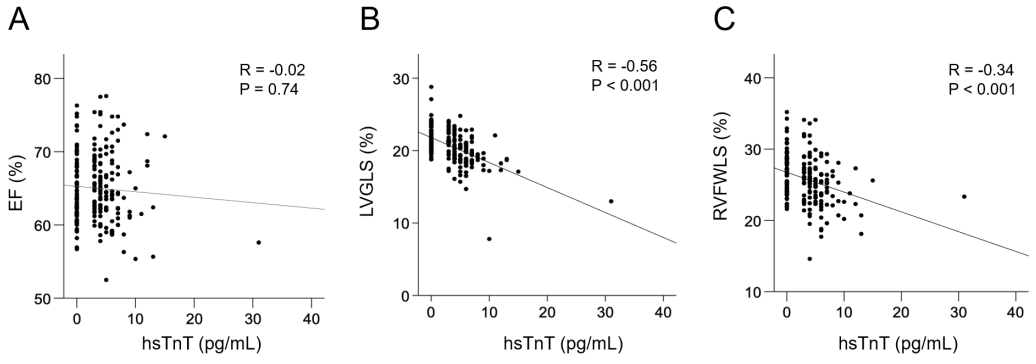

Figure 2. Relationship between hsTnT and cardiac function in recovered COVID-19 patients. There was no significant correlation between hsTnT and EF **(A)**. LVGLS **(B)** and RVFWLS **(C)** were significantly correlated with hsTnT. LVGLS had a more significant correlation with hsTnT than RVFWSL. EF, ejection fraction; hsTnT, high-sensitivity troponin; LVGLS, left ventricular global longitudinal strain; RVFWLS, right ventricular free-wall longitudinal strain.

the association of LVGLS and RVFWSL decline with the level of hsTnT values, including age, BMI, impaired renal function, diabetes, hypertension, and in-hospital oxygen demand as independent variables.

In the multiple regression analysis adjusted for LVGLS, age (β = -0.14, 95% confidence interval [CI] = -0.05 to -0.001, $p = 0.04$), male sex (β = -0.26, 95% CI = -1.78 to -0.62, $p < 0.001$), and elevated hsTnT (β = -0.34, 95% CI = -0.30 to -0.12, $p < 0.001$) were independent risk factors (Table 2).

In the multiple regression analysis adjusted for RVFWSL, age (β = -0.23, 95% CI = -0.11 to -0.02, $p = 0.005$), males (β = -0.15, 95% CI = -2.0 to -0.06, $p = 0.04$), and BMI (β = -0.19, 95% CI = -0.30 to -0.05, $p = 0.008$) were associated with RVFWSL. The association between hsTnT and RVFWSL was not statistically significant (β = -0.14, 95% CI = -0.28 to 0.02, $p = 0.08$) (Table 2).

Reproducibility

Excellent correlations were shown in the inter- and intra-observer variability of LVGLS, RVGLS, and RVFWLS ($r = 0.95$ and $r = 0.94$ for LVGLS, $r = 0.94$ and $r = 0.96$ for RV4CSL, and $r = 0.97$ and $r = 0.90$ for RVFWLS). Bland-Altman analysis showed that interobserver and intra-observer variability was -0.5 ± 1.8% and -0.5 ± 1.9 for LVGLS, 0.6 ± 2.6% and 0.6 ± 2.5% for RV4CSL, -0.3 ± 2.8% and 0.2 ± 1.5% for the RVFWLS.

Discussion

Of the 209 patients who recovered from COVID-19 infection, 67 (32.1%) had reduced LVGLS. Furthermore, LVGLS was associated with elevated hsTnT levels. These abnormalities were seen in patients in their late thirties, and their frequency increased with age. In our study, 65% of COVID-19 recovered patients had elevated hsTnT levels above detection sensitivity, whereas in a previous study conducted in Germany (*7*), 71% of recovered patients had positive hsTnT above detection sensitivity, and the frequency of myocardial dysfunction was similar to the frequency of positivity in our study.

Cardiac MRI studies have shown a correlation between hsTnT and positive T1 mapping, which can detect myocardial damage (*7*). Our echocardiographic studies also showed a significant correlation between LVGLS and hsTnT, suggesting that echocardiography, which is even easier to perform than cardiac MRI, can also detect small myocardial damage. A multicenter study reported that almost half of the patients admitted with COVID-19 had some abnormal echocardiographic findings, which influenced the changes in treatment strategy (*16*). Other multicenter studies have reported that elevated troponin and comprehensive echocardiographic abnormalities, including global LV dysfunction, wall motion abnormalities, diastolic dysfunction, RV dysfunction, and the presence of pericardial effusion, affected all-cause mortality (*10*). Although these past studies did not perform myocardial strain analysis, it indicated the importance of detecting abnormalities on echocardiography. In addition, it has been reported that abnormalities in LVGLS, RVLS, and TAPSE were independent predictors of in-hospital mortality in COVID-19 patients (*9,11*). Echocardiographic myocardial strain analysis is an accurate and reproducible imaging technique that is angle-independent (*17-19*). Global longitudinal strain (GLS) measured by echocardiography has been recognized as a more useful parameter than LVEF for detecting subtle abnormal changes and has been successful in predicting cardiovascular diseases (*14,20*).

In this study, 80% of patients were mildly ill with no history of oxygen inhalation, and on multivariate linear analysis, a history of oxygen inhalation was not associated with lower LVGLS. LVGLS has been reported to be affected by age, impaired renal function, diabetes mellitus, and hypertension (*21,22*). In addition, a validation study of echocardiographic myocardial strain due to aging in healthy subjects reported that LVGLS physiologically declined with age after 70 years of age (*23*). Our study did not include patients over 70 years of age or patients with a history of cardiovascular disease such as coronary artery disease, and strain validation in the control group showed an absolute value of more than 20% as normal, the same as guidelines and previous reports (*14,23*); therefore, the effects were minimal. We have shown that elevated hsTnT is an independent marker of myocardial damage even after adjusting for these risk factors in multiple regression analysis. Furthermore, even a small elevation above detection sensitivity is useful.

In our analysis, RV function was rarely less than 20%, as defined by the guidelines (*15*), during COVID-19 recovery, although RVGLS was significantly different in each group of hsTnT and was slightly less related to hsTnT in multiple regression analysis. However, a study of COVID-19 hospitalized patients in China found a predominant difference in mortality with RVGLS below 23% of the absolute value (*13*). In the extreme phase of COVID-19 infection, the cytokine storm causes damage to the right heart system due to acute respiratory distress syndrome (*24,25*) and pulmonary thromboembolism (*26-29*). In our study, the impairment of RVGLS was related to BMI even during COVID-19 recovery. It has been reported that COVID-19 is more severe in obese patients (*30*). Obesity may have an impact on the prolongation of RV dysfunction in COVID-19. More studies are needed on RV function during COVID-19 recovery.

Our study is the first to analyze both myocardial strain and high-sensitivity troponin at the same time in COVID-19 patients during recovery, and to assess the prevalence of myocardial damage as an aftereffect. In this study, there were few abnormalities in RV function, but LV dysfunction was present in about 30% of the

patients, and was also associated with elevated hsTnT levels, suggesting that myocardial damage may persist even in the recovery phase of COVID-19. In the future, during the recovery period, it will be important to screen for myocardial damage by echocardiographic reduction in LVGLS or an increase in hsTnT levels above detection sensitivity, followed by cardiac MRI to detect myocardial damage with a more detailed approach.

Our study has some limitations. This was a single-center study, data collection was retrospective, and there were no echocardiographic or hsTnT data before or during COVID-19 infection, and no cardiac magnetic resonance or cardiac catheterization data. There were no comparisons with a control population with cardiovascular risk factors for obesity or diabetes other than hypertension. As the analysis was based on stored serum samples, serological markers such as brain natriuretic peptide were not tested due to insufficient sample volume. There were variations in the time between recovery and testing, so in some cases, abnormalities may not have been detected. Other echocardiographic imaging protocols and post-processing approaches may yield different results; therefore, if multicenter studies are conducted in the future, speckle tracking measurements need to be adjusted for machine-to-machine errors. Finally, there were no outcomes such as cardiovascular events or death in this study, and a longer-term prospective study should be conducted in the future.

Conclusions

In this Japanese cohort study of recovered COVID-19 patients, even a slight increase in hsTnT was an independent marker of decreased LVGLS, and may thus be a useful marker of myocardial injury in recovered COVID-19 patients. Therefore, even if there are no obvious electrocardiogram or chest X-ray abnormalities in recovered COVID-19 patients, it is important to measure hsTnT, and in cases with detectable levels, measurement of LVGLS by echocardiography is recommended and the use of cardioprotective drugs might be considered.

Funding: This work was supported by a donation from the National Center for Global Health and Medicine and supported by the Health, Labour, and Welfare Policy Research Grants, Research on Emerging and Re-emerging Infectious Diseases and Immunization (grant number 20HA1006). The funders/sponsors have had no role in the design and/or implementation of the study.

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

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Received March 7, 2021; Revised March 24, 2021; Accepted March 29, 2021.

Released online in J-STAGE as advance publication April 2, 2021.

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Global Health & Medicine. 2021; 3(2):102-106.
COMMUNICATION
DOI: 10.35772/ghm.2020.01104

COVID-19 in Okayama Prefecture: Looking back and looking forward

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Abstract: In Japan, clinical and experimental studies addressing COVID-19 have been increasing in number since early February 2020, with many case reports being published. Concurrently, many notifications and guidelines have been issued from the government and academic societies. Taking optimal measures at the prefectural level as well as the national level is necessary to prevent the spread of COVID-19. Surveying and analyzing details of the incidences of infected persons in each prefecture is extremely important. This report describes the epidemiological characteristics of COVID-19 observed in Okayama Prefecture, followed by discussion of the direction of public health actions to be taken in the future. We reiterate the crucial importance of reinforcing and maintaining current public health measures, including rapid and detailed compilation of information related to infected persons and their surroundings, appropriate blocking of viral transmission, and early containment of infected persons, to minimize the spread of infection especially during the overlapping epidemic period of influenza in Okayama Prefecture.

***Keywords*:** coronavirus disease 2019 (COVID-19), epidemic period of influenza, epidemiological survey, novel coronavirus pneumonia, Okayama University Hospital, public health actions

Introduction

COVID-19 is transmitted mainly through droplet infection, which occurs without coughs or sneezes in a poorly ventilated environment. Contact infection might also occur through nasal discharge or saliva. Although symptomatic patients comprise most infection sources, a non-negligible risk of infection is posed by asymptomatic carriers. The latent period is 1-14 days. Symptoms usually appear around 5 days after exposure (*1*).

Although infected people younger than 50 years of age (including children) are usually asymptomatic or only mildly symptomatic, the disease is more threatening and not infrequently fatal to people who are 60 years old or older. Patients might develop more severe symptoms when they have underlying diseases such as chronic respiratory disease, cardiovascular disease, hypertension, diabetes, severe obesity, hematologic tumor, and immunodeficiency.

The period during which infection can be transmitted to another person is regarded as being from 2 days before onset to 7-10 days after onset of symptoms. Not a small number of infected people have already infected other people by the time of onset. The viral level in the respiratory tract, along with high infectiousness, is also high in asymptomatic persons with infection. Viral excretion is presumed to peak from 1 day before onset to the day of onset. It is likely that an infected person will spread infection with no knowledge of doing so. This characteristic differs from influenza (*2*).

Looking back on COVID-19 in Japan

In December 2019, an outbreak of novel coronavirus (severe acute respiratory syndrome coronavirus 2: SARS-CoV-2) pneumonia was reported in Wuhan, Hubei Province, China. Later, as the infection spread worldwide, the World Health Organization (WHO) declared a public health emergency on 30 January 2020. The first infected person in Japan was reported on 16 January 2020. COVID-19 was defined as a designated infectious disease by government ordinance on 1 February. The WHO declared COVID-19 a pandemic on 11 March 2020.

What actions were taken by the Government of Japan and the Ministry of Health, Labour and Welfare at this early stage of the pandemic? Under those circumstances, the Government of Japan declared a state of emergency from 16 April through 25 May for all prefectures. The government recommended that the nation adopt a lifestyle that includes reduction of contact with people by approximately 80%. It appears that a remarkable "lockdown"-like effect was achieved through the active participation of people. Apparently, Japan has

successfully controlled the numbers of infected persons and reduced the deaths to low levels in this way without legal restrictions (*3*).

New incidences of SARS-CoV-2 infection in Japan were markedly suppressed for approximately two months from late April through late June. However, the number of persons with infection increased rapidly thereafter. According to Japan's Ministry of Health, Labour and Welfare, a cumulative total of 78,847 persons with infection and 1,511 deaths had been confirmed in Japan as of 22 September 2020.

Looking back on COVID-19 in Okayama Prefecture and Okayama University Hospital

Japan experienced two waves of imported COVID-19 cases, after which local transmission occurred and the epidemic grew. In Okayama Prefecture, the first infected person was reported on 22 March 2020 in Okayama City. By 11 May 2020, the total number of COVID-19 cases reached 25 (forming the "first wave"). After a period with no new infection for 44 days, another infected person was identified on 24 June 2020 followed by an increasing number of persons with infection (forming the "second wave"). The Governor of Okayama Prefecture issued a strong message to people inside and outside the prefecture, asking them to "please refrain from moving across prefectural borders" before the long holiday from April to May at the early stage of the pandemic. The majority of the local people seemed to continue practices of universal masking and social distancing following the message, thereby contributing to the low number of infected persons in Okayama Prefecture.

The Okayama University Hospital is the designated medical institution for class I infectious diseases. It has been led under the strong leadership of its Director, who has vowed a policy of "Do not let patients die in Okayama Prefecture." The hospital has been holding morning meetings, attended by the medical counselor of the Okayama Prefectural Government, every week since 5 March 2020 and making immediate decisions on policy. The Director and eight assistant directors, including the corresponding author of this paper, have been discussing and sharing information about the medical care system in remote meetings with directors of major general hospitals, including three designated medical institutions for class II infectious diseases, in the prefecture every week since 8 April 2020. The chief of the health promotion section of the Okayama Prefectural Government and the director of the Okayama Healthcare Center also attended the meetings (*4*).

Various manuals have been prepared, and revised as appropriate, mainly by the Infection Control Team of the hospital. Medical doctors in the Infection Control Team have been on call 24 hours a day since the middle of March 2020. Those doctors joined the cluster management team of the Okayama Prefectural Government and the contact tracing team of the Okayama Healthcare Center. As a result, our hospital has been in close cooperation with the Okayama Prefectural Government and the Okayama Healthcare Center.

Our hospital is the "last defense" against severe diseases in the Chugoku and Shikoku regions. For COVID-19 we conduct systemic management of patients in critical or severe conditions who will need advanced facilities, including extracorporeal life support. The other general hospitals provide medical care to patients mainly in moderate or mild conditions (*4*).

Epidemiological survey on COVID-19 in Okayama Prefecture

Actions suitable for the healthcare system of each prefecture are necessary to prevent the spread of COVID-19. For that purpose, surveying and analyzing details of the incidences of persons with infection in each prefecture is extremely important. However, reports have been insufficient to date. Recently, our group conducted a detailed survey of incidents of persons with SARS-CoV-2 infection in Okayama Prefecture and analyzed the current situation in Okayama, forming the basis for discussions on the direction of public health actions to be taken in the future (*5*).

The survey was performed using records from the Okayama Prefecture website and data from Sanyo Shimbun (digital version). We defined the period of the "first wave" as extending from 22 March through 11 May 2020, the "second wave" as extending from 24 June through 22 September 2020 and divided subjects into these two groups because no infections were reported (for 44 days) from 12 May through 23 June 2020. The last day of the survey has been extended from 24 August 2020 in Higashionna's paper (*5*) to 22 September 2020 in the present paper. The essential points of the results are summarized below:

i) The first wave and second wave included 25 patients and 123 patients, respectively. They included 15 males (15/23, 65%) among 23 cases in the first wave, and 67 males (67/117, 57%) among 117 cases in the second wave, excluding unknown cases (2 in the first wave, 6 in the second wave).

ii) Excluding unknown cases (1 in the first wave, 7 in the second wave), infected persons in their 50s accounted for 29% of the first wave and comprised the largest age group. Persons aged 50 years and older accounted for 54% of the total. In the second wave, infected persons in their 20s accounted for 41% which was the largest, followed by 30s for 13% and 40s for 16%. Persons aged 20-49 accounted for 70% of the total.

iii) Regarding the residence of persons infected with SARS-CoV-2, the locations with greater numbers were

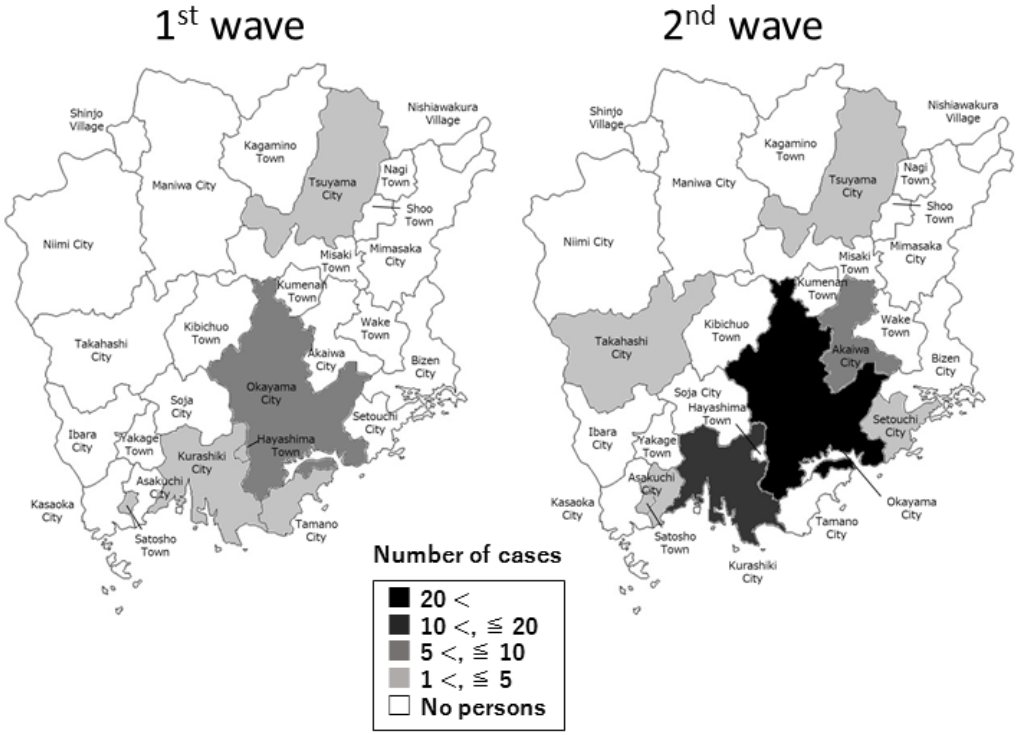

Figure 1. Geographic distribution of cases of SARS-CoV-2 infection (COVID-19) during the first and second waves in Okayama Prefecture.

Okayama, Hayashima, and Tsuyama in the first wave, and Okayama, Kurashiki, and Akaiwa in the second wave, in descending order. Okayama City residents accounted for 65% and 72% of the total in the first wave and second wave, respectively (Figure 1).

iv) Investigation excluding cases with unknown epidemiological link (6 persons in the first wave, 73 persons in the second wave) showed that infection between persons both aged at least 20 years occurred in 4 persons in the first wave, and in 47 persons in the second wave. One person in the first wave and 4 persons in the second wave were aged below 20 years and were infected from persons aged at least 20 years. All of these 5 persons were between 10 and 20 years of age. There were no cases of anyone under 20 years of age infecting anyone else.

v) No cluster was found in the first wave, although three clusters were identified in the second wave. All clusters occurred in Okayama City. The numbers of infected persons in the clusters were 5, 8, and 6, making 19 in all.

vi) For cases in which a person in close contact was positive, excluding cases with an unknown epidemiological link (sporadic cases, or the first case when infection spread in a specific group, or cases with unknown details), the time from the date on which the first case was confirmed positive by PCR to the date when a person in contact was confirmed positive by PCR (designated as "time to positive PCR") was surveyed. The mean time to positive PCR ± SD / median (minimum - maximum) was 0.8 ± 0.8 days /1 (0-2) days in the first wave (6 persons in total), and 2.0 ± 1.4 days /2 (0-9) days in the second wave (73 persons in total).

Looking forward on COVID-19 in Okayama Prefecture

Japan comprises 47 administrative districts (prefectures), covering an area of 377,900 km^2 and supporting a population of 125.7 million (population density: 333 persons/km^2). Okayama Prefecture, located in the southern part of the Chugoku region, faces the Seto Inland Sea, covers an area of 7,114 km^2, and has a population of 1.88 million (population density: 264 persons/km^2).

The cumulative number of persons infected with SARS-CoV-2 as of 22 September 2020 was 148 including 1 person who tested positive again. The cumulative number was the second highest in the Chugoku and Shikoku regions following Hiroshima. However, the number of infected persons per 100,000 people was 7.8, which was the second lowest in the Chugoku and Shikoku regions, next to 6.5 in Tottori. Although cities and towns in the Chugoku and Shikoku regions are connected to urban areas such as Tokyo, Nagoya, and Osaka via the Okayama City traffic hub (JR Okayama station on the Sanyo Shinkansen line), the number of infected persons per population was remarkably smaller at the prefectural than the national level (62.4 per 100,000 people) (*https://web.sapmed.*

ac.jp/canmol/coronavirus/japan).

As shown in Figure 1, the incidence of infected persons in Okayama Prefecture tended to spread concentrically, centered on Okayama City in both the first wave and the second wave. In the future, strict infection control in Okayama City is expected to decrease the incidence of infection throughout Okayama Prefecture.

The percentage of infected persons aged 20 through 40 was higher in the second wave compared to the first wave. This trend matched the overall trend in Japan (*1*). However, of national concern is that infection might spread more among elderly people than young people in the near future. Reportedly, mortality in infected elderly people is much (over 100 times) higher than in infected young people. For that reason, the incidence by age group continues to attract attention (*https://vdata.nikkei.com/newsgraphics/coronavirus-japan-chart*).

Five persons in all were younger than 20 years (but older than 10) and had been infected by people aged 20 years or older. There were no reports of any persons under 20 years of age infecting anyone else. In Okayama Prefecture, it is estimated that the percentage of people below age 20 involved in infection transmission is rather small. It is intriguing that children in general are less susceptible to severe COVID-19 (*6*).

Three incidences of SARS-CoV-2 cluster infections were observed, with a total of 19 patients in the second wave only. These occurred in entertainment establishments that serve beverages or food in Okayama City. The spread of infection was limited to a small scale. Presumably, a subsequent rapid increase in the number of infected persons was prevented by the following factors: surveys of persons in close contact, led by public health centers, were conducted rapidly; establishments where clusters occurred actively participated in surveys of surrounding persons; and the prefectural governor of Okayama emphasized and promoted the importance of compliance to the "New Lifestyle".

From the viewpoint of the "time to positive PCR", we can infer that public health centers in Okayama Prefecture responded rapidly. To prevent the subsequent spread of infection, it is important to rapidly provide instructions for infection prevention and conduct PCR testing for close contacts of infected persons at the appropriate time. Thorough surveys of persons in contact, assisted by the recently intensified PCR test system have led to the prevention of infection spread during the second wave in Okayama Prefecture.

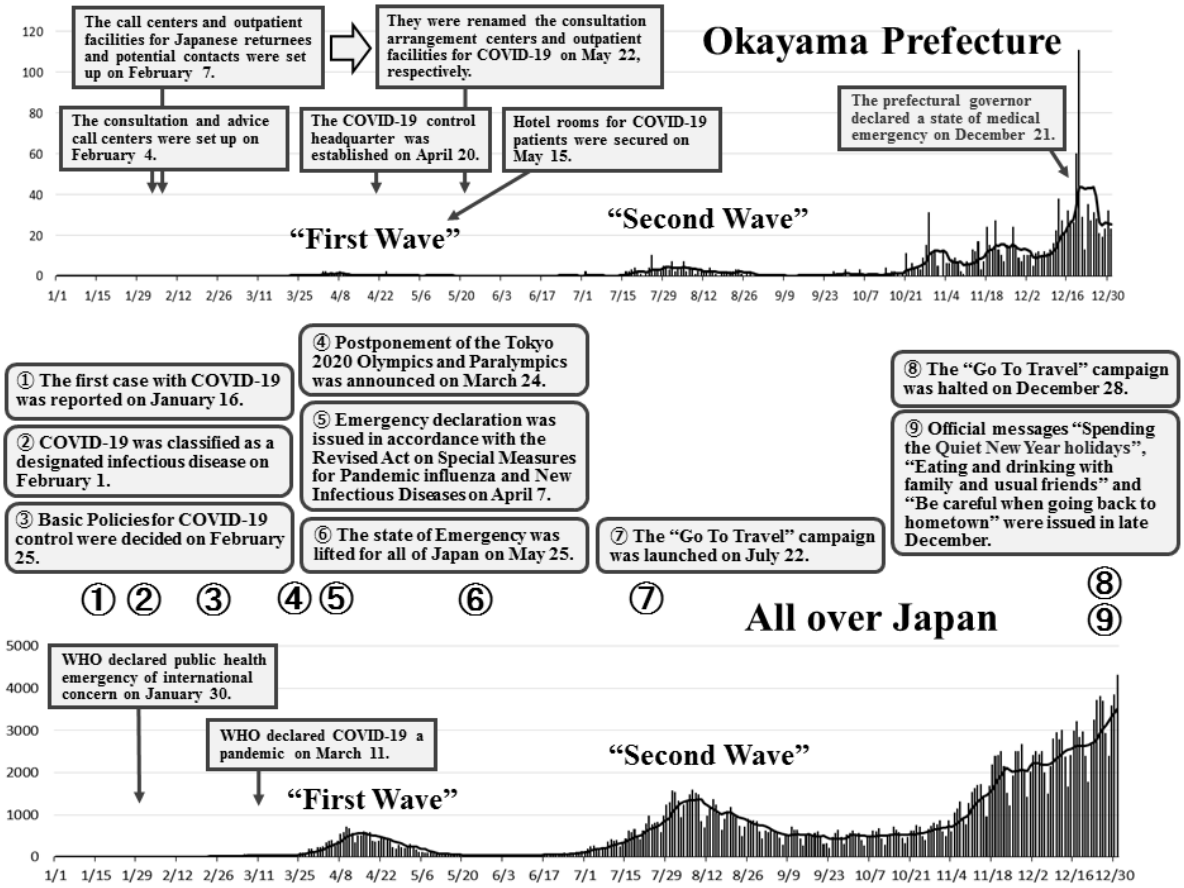

Figure 2. Confirmed cases of COVID-19. Daily numbers and 7-day averages of confirmed cases are plotted by reported date (January 16, 2020 to December 31, 2020) in Okayama Prefecture (top panel) and all over Japan (bottom panel). Major epidemic response actions taken by the Japanese Government (from ① to ⑨) are shown.

Future direction of public health action in Okayama Prefecture

Just before the submission of the first draft of our manuscript, we found that several prefectures in Japan were experiencing a resurgence of COVID-19 cases (*7*). Around late October, several clusters of COVID-19 cases occurred mostly in non-urban areas in Okayama Prefecture. Apparently, the virus resurged along with resumption of socioeconomic activities.

As of 31 December 2020, the number of confirmed cases in Japan reached 232,495, with 3,459 deaths (fatality rate: 1.5%) (*https://www.mhlw.go.jp/stf/newpage_15831.html*), whereas the number of confirmed cases in Okayama Prefecture reached 1,363, with 15 deaths (fatality rate: 1.1%) (*https://www.pref.okayama.jp/page/700024.html, https://www.pref.okayama.jp/page/667843.html#shibou*). Therefore, Okayama Prefecture can still be regarded as a region that is not severely affected by COVID-19 (Figure 2).

The Okayama University Hospital has treated 38 patients with COVID-19. Most of them were in critical or severe clinical condition, with two patients subsequently requiring extracorporeal life support. Their ages ranged from 26 to 90 years, with a median age of 60 years (mean ± SD: 57 ± 20); and 20 (53%) were male. No patient has died at our hospital. As additional information, we report that the Emergency Rescue Team of the hospital has developed the portable transparent vinyl chloride shield as a feasible tool to securely cover the face of the COVID-19-positive patient during emergency transport (*8*).

To prevent the spread of COVID-19, the National Institute of Infectious Diseases emphasizes the importance of taking action at the national and prefectural (not just local) levels. Considering the mode of transmission of SARS-CoV-2, high prevalence of COVID-19 is expected to prevail during and after November 2020, particularly because of its overlap with the epidemic period of influenza (*2*). It is critical to continue efforts in containment of SARS-CoV-2 transmission at an early phase, thereby simultaneously preventing infection spread and maintaining socioeconomic activities.

In conclusion, we infer the crucial importance of reinforcing and maintaining current public health measures including rapid and detailed comprehension of information related to infected persons and their surroundings, appropriate blocking of viral transmission, and early containment of infected persons. By doing so, it will be possible to minimize the infection spread and overlapping of the epidemic period of influenza in Okayama Prefecture, where the numbers of cases and deaths are fewer than those of more densely populated prefectures such as Tokyo, Osaka, Kanagawa, Aichi, and Saitama.

Funding: None.

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

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Received November 23, 2020; Revised January 15, 2021; Accepted January 22, 2021.

Released online in J-STAGE as advance publication March 6, 2021.

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Global Health & Medicine. 2021; 3(2):107-111.
COMMUNICATION
DOI: 10.35772/ghm.2020.01109

Effective screening strategies for detection of asymptomatic COVID-19 travelers at airport quarantine stations: Exploratory findings in Japan

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Abstract: The quantitative reverse transcription polymerase chain reaction method using nasopharyngeal swabs (NPS RT-qPCR) is regarded as the reference standard for diagnosing coronavirus disease 2019 (COVID-19). However, when using NPS RT-qPCR at busy airport quarantine stations, there are constraints on testing capacity, time, travelers' tolerance, and availability of personal protective equipment for quarantine officers. A feasible alternative is therefore needed to test incoming travelers, especially when passenger numbers increase with the resumption of business, tourism, and economic activities. To explore alternatives to NPS RT-qPCR, we collected nasopharyngeal, anterior nasal, and saliva samples chronologically over days 1-7 from asymptomatic COVID-19 air travelers who were under quarantine at a designated facility, and we then compared test results for 9 different methods, comprising RT-qPCR (including the reference method), loop-mediated isothermal amplification (LAMP), and qualitative and quantitative antigen testing. We evaluated sensitivity for 97 person-day samples independently to evaluate asymptomatic travelers regardless of their testing date and period of asymptomatic status upon entry. Sensitivity of the different tests varied from 46.6% to 81.0%, but this was improved from 72.7% to 100.0% when the viral load was > 10^4 copies/sample on NPS RT-qPCR. Thus, most high-risk asymptomatic travelers with higher viral load would be detected by the tests evaluated. Quantitative antigen testing using saliva samples showed 90.9% sensitivity and provided quicker results, and should be an acceptable alternative to NPS RT-qPCR at busy airport quarantine stations. We discuss the implications of our exploratory findings for establishing a comprehensive and feasible testing strategy for COVID-19 among air passengers.

***Keywords*:** *in vitro* diagnostics, SARS-CoV-2, PCR, antigen testing, saliva, point of entry

Introduction

The global novel coronavirus disease 2019 (COVID-19) pandemic has led most countries to implement some form of travel restrictions, health screening and quarantine measures (*1*). In Japan, after COVID-19 was designated a quarantinable infectious disease on February 1, 2020 (*2*), quarantine officers started testing for symptomatic and suspected cases of COVID-19 infection. On March 9, entry restrictions became stricter, with quarantine measures strengthened to include testing even asymptomatic travelers by quantitative reverse transcription polymerase chain reaction (RT-qPCR) or loop-mediated isothermal amplification (LAMP) using nasopharyngeal swab samples (NPS), and isolation for COVID-19 positive travelers.

As asymptomatic carriers can unknowingly infect others, especially during the 2-3 days before symptom onset (*3*), all incoming travelers should be tested at the point of entry. Among the nucleic acid amplification testing (NAAT) methods available to use, NPS RT-qPCR is the reference standard. However, when conducted at quarantine stations, the results can take a long time, swab collection may cause travelers discomfort and bleeding, and quarantine officers are at risk of exposure, requiring personal protective equipment (PPE) to be worn. An alternative strategy is therefore needed for busy quarantine stations, particularly as economic and business activities resume and numbers of inbound travelers increase.

Several diagnostic tests for SARS-CoV-2 are approved for use in Japan (*4*), but there is no consensus or definitive guidance on the most effective method for mass screening of travelers. As all testing strategies have advantages and disadvantages, we felt it prudent to evaluate the feasibility of using alternatives to the reference standard NPS RT-qPCR. Therefore, in this study, we compared the sensitivity and feasibility of

different tests using different samples against that of NPS RT-qPCR, by testing samples obtained from travelers confirmed positive for SARS-CoV-2 at airport quarantine. We report here our exploratory findings and suggest points to note when applying screening strategies with inbound travelers at airports.

Sample collection and evaluation with 9 different testing methods

Following approval by the Institutional Review Board of the National Center for Global Health and Medicine (NCGM-G-003641-00), we prospectively collected samples from inbound travelers to Japan who tested positive on NPS RT-qPCR or NPS LAMP at either of Tokyo's two international airports (Narita or Haneda) between July 27 and August 1, 2020. Of 7,689 passengers (from 283 commercial flights) whose samples were tested at the quarantine laboratory, 51 were positive for SARS-CoV-2. The 27 travelers diagnosed as COVID-19 asymptomatic carriers and transferred to a COVID-19 quarantine facility were eligible for this study (Figure S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=17*).

Full details of the inclusion criteria, sample collection, SARS-CoV-2 detection, and statistical analysis are provided as Supplementary Data (Methods S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=17*). Briefly, from day 1 until day 7 under quarantine, each participant was asked to provide a complete set of 4 self-collected samples and 2 physician-collected samples (NPS) that were simultaneously obtained for testing on the same day. The self-collected samples were 2 saliva samples and 2 dry swab samples taken from the anterior 2/3 of the dorsum of the tongue and the anterior nasal cavity. The 2 physician-collected samples were NPS samples, taken with a dry swab and a flocked swab. Samples were collected between July 27 to August 8, 2020, at which time the national quarantine measures changed. In that time frame, 20 quarantined individuals agreed to participate: most participants were male (85%), age < 40 years (70%), most embarked in the Philippines (45%), and 75% were seamen with special entry permission due to imminent departure from Japan by ship (Table 1). The number of participants who provided a complete set of samples every day was 4 for 7 days, 8 for 6 days, 2 for 5 days, 1 for 4 days, 1 for 3 days, and 4 for 1 day, yielding 97 person-day samples in total.

Samples were tested using the following 9 methods for detecting SARS-CoV-2 (Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=17*) at independent facilities (see Methods S1 for details, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=17*), with sensitivity and 95% confidence intervals (CI) calculated to assess the diagnostic performance of each test. Cohen's kappa (k) coefficients were then calculated to determine the concordance rate between the results of *i*) reference-standard NPS RT-qPCR and *ii*) RT-qPCR using the anterior nasal swab sample (anterior nasal RT-qPCR); *iii*) RT-qPCR using a saliva sample (saliva RT-qPCR); *iv*) direct RT-qPCR using a saliva sample (saliva direct RT-qPCR); *v*) LAMP using a saliva sample (saliva LAMP); *vi*) RT-qPCR using the tongue swab sample (tongue RT-qPCR); *vii*) quantitative antigen testing using a saliva sample (quantitative saliva antigen); *viii*) quantitative antigen testing using an NPS sample (quantitative NPS antigen); and *ix*) qualitative rapid antigen testing using an NPS sample (qualitative NPS antigen; rapid antigen test). We used the cutoffs for quantitative antigen testing of 0.67 pg/mL for saliva and 1 pg/mL for NPS, according to the manufacturer's instructions.

Table 1. Baseline characteristics of 20 international travelers diagnosed as asymptomatic carriers of COVID-19 at two airport quarantine stations in Tokyo (July 27-August 1, 2020)

Variables	Number	(%)
Sex		
Male	17	(85)
Female	3	(15)
Age, years		
20-29	5	(25)
30-39	9	(45)
40-49	5	(25)
50-59	1	(5)
Country of embarkation		
Philippines	9	(45)
India	3	(15)
Bangladesh	3	(15)
United Kingdom	1	(5)
Mexico	1	(5)
Pakistan	1	(5)
France	1	(5)
Ukraine	1	(5)
Occupation		
Seaman	15	(75)
Office worker	2	(10)
Coordinator	1	(5)
Merchant seaman	1	(5)
Unemployed	1	(5)
Comorbidity		
None	20	(100)
Smoking status		
Current smoker	2	(10)
Ex-smoker	6	(30)
Never smoker	12	(60)
Symptomatic status on entry[a]		
Asymptomatic	14	(70)
Pre-symptomatic	3	(15)
Post-symptomatic	2	(10)
Pre- and post-symptomatic	1	(5)

[a]Symptomatic status was defined as follows: asymptomatic, asymptomatic before and upon entry to Japan; pre-symptomatic, developed symptoms during quarantine; post-symptomatic, symptoms had appeared before entry; and pre- and post-symptomatic, symptoms had appeared before and after entry.

Differences found between the various testing methods

We evaluated sensitivity for the 97-person-day samples independently in order to evaluate the asymptomatic travelers regardless of their testing date and period of

Table 2. Cohen's Kappa coefficients (κ) and sensitivity for various tests compared with reference-standard NPS RT-qPCR (97 samples from 20 quarantined travelers diagnosed with COVID-19)

Items	Cohen's kappa	Sensitivity (%)		
		All	Viral load ≥ 10^4 copies/sample	Viral load < 10^4 copies/sample
Self- collected samples				
Anterior nasal RT-qPCR[a]	0.56	69.0	100	28
Saliva RT-qPCR[b]	0.50	63.8	100	16
Saliva direct RT-qPCR[c]	0.39	46.6	81.8	0
Saliva LAMP[d]	0.51	60.3	100	8
Tongue RT-qPCR[a]	0.31	44.8	72.7	8
Quantitative saliva antigen testing[e]	0.46	55.2	90.9	8
Physician-collected samples				
Quantitative NPS antigen testing[e]	0.41	81.0	100	16
Qualitative NPS antigen testing[f] (rapid antigen test)	0.55	60.3	100	8

[a] cobas® SARS-CoV-2 (Roche Diagnostics, Indianapolis, IN). [b] Primer and probe set recommended by Japan's National Institute of Infectious Diseases (*13,14*). [c] SARS-CoV-2 Direct Detection RT-qPCR Kit (Takara Bio, Kusatsu, Japan). [d] Loopamp® 2019-SARS-CoV-2 Detection Reagent Kit (Eiken Chemical, Tokyo, Japan). [e] Lumipulse® G1200 system and Lumipulse SARS-CoV-2 Ag kit (Fujirebio, Tokyo, Japan). [f] ESPLINE SARS-CoV-2 rapid antigen test (Fujirebio). NPS, nasopharyngeal swab; RT-qPCR, quantitative reverse transcription polymerase chain reaction.

asymptomatic status upon entry. Table 2 shows Cohen's kappa coefficients and sensitivity results for the 8 tests compared with NPS RT-qPCR. NAAT showed anterior nasal RT-qPCR had the highest sensitivity (69%, 95% CI: 55.5-80.5), followed by saliva RT-qPCR (63.8%, 50.1-76.0) and saliva LAMP (60.3%, 46.6-73.0), with low sensitivity seen for saliva direct RT-qPCR (46.6%, 33.3-60.1) and tongue RT-qPCR (44.8%, 31.7-58.5). Concordance between the tests was generally moderate but was low for tongue RT-qPCR (0.31, 0.13-0.49) and saliva direct RT-qPCR (0.39, 0.22-0.67). On quantitative NPS and saliva antigen testing, sensitivity was 55.2% (41.5-68.3) for saliva and 81.0% (68.6-90.1) for NPS. On qualitative NPS antigen testing, sensitivity was 60.3% (46.6-73.0).

When viral load was > 10^4 copies/sample for targets 1 and 2 on NPS RT-qPCR (33 samples), sensitivity was improved to 100% for anterior nasal RT-qPCR (95% CI: 84.7-100), saliva RT-qPCR (84.7-100), quantitative NPS antigen (cut-off ≥ 1 pg/mL, 84.7-100), saliva LAMP (84.7-100), and qualitative NPS antigen (84.7-100) and to 90.9% for quantitative saliva antigen (75.7-98.1), 81.8% for saliva direct RT-qPCR (64.5-93.0), and 72.7% for tongue RT-qPCR (54.4-86.7). The detailed test results for all 20 participants are shown in Table S2 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=17*).

Possible implications of our exploratory findings

Overall, some clear differences were evident between the 8 testing strategies compared with NPS RT-qPCR over days 1 to 7, as determined by three independent laboratories, for asymptomatic international travelers who tested positive for SARS-CoV-2 on arrival at the airport. We have two major findings. First, compared with NPS RT-qPCR, the 8 tests showed varied sensitivity (44.8%-81.0%) and Cohen's kappa coefficients (0.31-0.56), and the test results for tongue RT-qPCR, saliva direct RT-qPCR, and quantitative saliva antigen testing showed lower sensitivity (44.8%, 46.6%, and 55.2%, respectively) than in previous studies (*5-7*). Second, the sensitivity of tongue RT-qPCR, saliva direct RT-qPCR, saliva RT-qPCR, and quantitative saliva antigen testing was improved (72.7%, 81.8%, 100%, and 90.9%, respectively) among participants showing a high viral load on NPS RT-qPCR.

Test sensitivity has varied across settings. NPS and saliva RT-qPCR showed highly consistent results in a mass-screening study in Japan of asymptomatic individuals from an airport quarantine group (n = 161) and a contact tracing group (n = 1,763); saliva RT-qPCR showed 92% sensitivity and 99.96% specificity (*5*). In American studies, estimated sensitivity was also high for tongue, nasal, and mid-turbinate RT-qPCR (89.8%, 94.0%, and 96.2%, respectively) compared with NPS RT-qPCR (*6*), although saliva RT-qPCR showed around 30% lower sensitivity relative to NPS samples in a diagnostic cohort and around 50% lower sensitivity in a convalescent cohort in a community setting (*7*). Possible reasons for saliva showing lower sensitivity in our study include the following. First, the results of saliva tests are more likely to be affected than NPS tests by unobserved self-collection of saliva samples and the oral cavity environment. Our participants self-collected samples unobserved while following instructions because we wanted to explore the feasibility of using this quick, easy, and well-tolerated saliva sampling method at busy airport quarantine stations. We asked participants to refrain from eating, drinking, chewing gum, and smoking for 1 h before saliva collection because these and similar activities may prevent SARS-CoV-2 detection in saliva (*8*). Thus, if saliva sampling is used at airports, cabin crew should remind passengers of these instructions well before landing. Also, self-collected saliva samples showed lower sensitivity than self-collected anterior

nasal swabs, possibly because the latter were collected under observation. Second, the collection method, timing, storage, and processing of saliva samples is not standardized worldwide (*9*). Sensitivity may have been reduced with our collection methods compared with, for example, drooling into a tube or using a pipet. Third, we targeted asymptomatic passengers and collected samples over days 1 to 7, which included participants found to be in the convalescent stage during the study period. Asymptomatic individuals were previously found to be less likely than symptomatic individuals to have detectable SARS-CoV-2 on NPS RT-qPCR (*10*), and our study did show lower sensitivity in the samples with lower viral load. Because asymptomatic carriers, in addition to symptomatic passengers, comprise the target population at airport quarantine stations, these four considerations should be kept in mind when evaluating the feasibility of saliva testing.

When evaluating the 9 different testing strategies for mass screening at busy airports, we considered sensitivity over time, speed, ease, and tolerability of sample collection. Saliva, tongue, and anterior nasal samples were, however, quicker and often more tolerable for travelers to provide than NPS samples (Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=17*). Antigen testing provided quicker results than NAAT (Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=17*), which is advantageous in a quarantine setting. We also evaluated self-collection of samples to reduce quarantine officers' exposure and PPE needs. For self-collected samples, saliva spit directly into a sterile tube showed more reliable results, and quarantine officers can visually confirm whether the sample is collected appropriately. Tongue and anterior nasal swabs are easy to self-collect, but quarantine officers should observe collection, so these methods are not suitable for busy quarantine stations.

From August 2020, based on a previous study (*5*), Japanese quarantine stations replaced NPS RT-qPCR or LAMP with quantitative saliva antigen testing for screening asymptomatic carriers (*11*). While we found that quantitative saliva antigen testing detected most asymptomatic carriers with higher viral load (90.9% sensitivity), some travelers with lower viral load will test negative. When screening asymptomatic travelers, who have lower pretest probability (positive rate 0.66% in this study at airport), not all asymptomatic carriers will be detected by point of entry testing. Negative results can create a false sense of security, so quarantine officers could provide travelers with accurate information about testing, including limitations, and still encourage essential preventive measures.

For a comprehensive quarantine strategy, travelers who test positive on point of entry testing should naturally isolate, but also all negative travelers should routinely self-quarantine, avoid public transport, and undergo health monitoring for 14 days. To date, this has been successful, with surveillance systems in Japan having found no large clusters in the community involving inbound travelers.

Using saliva samples in screening

Quantitative saliva antigen testing showed 90.9% sensitivity and provided relatively quick results, and should be an acceptable alternative to NPS RT-qPCR at busy airport quarantine stations. The points to note if using saliva samples to detect asymptomatic carriers are to *i*) remind passengers well before and upon landing to avoid eating, drinking, gargling, and smoking; *ii*) give appropriate instructions for saliva collection in order to standardize procedures; and *iii*) develop systems for digitalized health monitoring, contact tracing, and healthcare consultations that respect inbound travelers' privacy, regardless of infection status.

Future study

In this exploratory study, we were not able to obtain definitive results about sensitivity and specificity. Also, we recruited only NPS RT-qPCR-positive travelers detected at airport quarantine stations, so we could not evaluate specificity for each testing procedure, as calculations for the specificity of each test should also include RT-qPCR-negative travelers. Other studies have also been relatively small so far, with 48 samples analyzed from 48 patients in a hospital setting (*12*) and 30 samples from 30 travelers quarantined with mild SARS-CoV-2 infection (*10*). We hope that reporting our exploratory findings here can inform the design of a larger multicenter study to examine feasible alternatives.

Acknowledgments

We thank Toyomitsu Tamura, Tomoko Komagata, Shikino Kikuchi, Yui Ito, and Toshiaki Baba at the National Center for Global Health and Medicine for assistance with testing and preparation for study, Hiroshi Ohtsu for advice on statistical analysis, and Caryn Jones of ThinkSCIENCE (Tokyo, Japan) for assistance with writing and editing the manuscript.

Funding: This study was supported by grants from the Ministry of Health, Labour and Welfare, Japan (Health, Labour and Welfare Policy Research Grant 20HA2002).

Conflicts of interest: The authors have no conflicts of interest to disclose.

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Received December 9, 2020; Revised February 5, 2021; Accepted February 26, 2021.

Released online in J-STAGE as advance publication March 9, 2021.

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Global Health & Medicine. 2021; 3(2):112-114.

LETTER

DOI: 10.35772/ghm.2020.01079

Combating COVID-19 as a designated hospital: Experience from Shanghai, China

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Abstract: Many large international cities, such as Shanghai, are facing the threat of more imported cases of COVID-19 because of the frequent flow of people and objects at home and abroad. In the face of the complex and changing disease status of the international community, dealing with this disease effectively is a great challenge to the city's existing public health emergency response capacity and also a major test of designated COVID-19 hospitals. Here, we share our experience as a designated COVID-19 hospital in Shanghai, China in terms of *i*) A Professional Multi-disciplinary Team, *ii*) Personalized Treatment Plans for Patients in Severe or Critically Ill Condition, *iii*) Well-organized Classification of Patients, *iv*) Establishment of Transitional Wards, *v*) Nosocomial Infection Prevention and Control, and *vi*) Identification and Reporting of the Asymptomatic in the hopes that these approaches can serve as a reference for healthcare providers and medical staff who are fighting the pandemic.

***Keywords*:** COVID-19, designated hospital, treatment, asymptomatic

In January 2020, an outbreak of a new coronavirus pneumonia occurred in Hubei Province, China and spread to most parts of the country and the world (*1*). Shanghai, a large international city, is facing the threat of more imported cases because of the frequent flow of people and objects at home and abroad. In the face of the complex and changing disease status of the international community (*2*), dealing with this disease effectively is a great challenge to the city's existing public health emergency response capacity and also a major test of designated COVID-19 hospitals (*3*).

Following the SARS outbreak in May 2003, the Shanghai Municipal Government moved the Shanghai Infectious Disease Hospital to the southwestern suburbs of the city, where it was renamed the Shanghai Public Health Clinical Center (SPHCC). On November 16, 2004, the SPHCC was officially completed and began operations. In recent years, the SPHCC has played a significant role in responding to outbreaks such as H1N1, H7N9, Ebola, and MERS. During the COVID-19 outbreak, the SPHCC sent medical experts to the WHO and other countries to provide Chinese experiences and expertise. Our medical experts have also been involved in the revision and updating of clinical management guidelines for COVID-19 to facilitate the treatment and further control of COVID-19.

The ability to provide optimal clinical treatment is the basis for responding to public health outbreaks and for successfully treating patients (*4*). In the "downtime mode", the SPHCC has established specialized clinical services; the Center currently has 40 clinical departments and 7 medical technology departments. The scope of diseases treated at the SPHCC has expanded from traditional infectious diseases to comprehensive diagnosis and treatment of infectious diseases in particular, and the population served has gradually expanded. In the "active mode" during a public health emergency, the SPHCC has created negative pressure rooms with 327 beds in Area A (areas for patients with infectious diseases of the respiratory tract, such as COVID-19) and it has formed a professional medical treatment team. Patients with other diseases are transferred to Area B (areas for patients with infectious diseases of the digestive tract, such as hepatitis) or other areas for treatment.

Here, we share our experience as a designated COVID-19 hospital in the hopes that it will serve as a reference for healthcare providers and medical staff who are fighting the pandemic.

i) *A Professional Multi-disciplinary.* Team There was a severe shortage of medical resources early on during the outbreak (*5,6*), so the Shanghai Municipal Government coordinated the forming of a professional multi-disciplinary team that included all experienced medical experts in Shanghai specializing in infectious diseases, respiratory intensive care, intensive care, cardiothoracic surgery, and traditional Chinese medicine as well as nutritionists, rehabilitation physicians, psychologists, clinical pharmacists, and laboratory

physicians. The team has been stationed at the SPHCC and helped to treat patients with COVID-19. Depending on the number of patients admitted and the proportion in severe condition, a plan for medical workers needed is formulated. Thus far, a total of 641 front-line medical staff have helped to treat patients with COVID-19 at the SPHCC. In addition, 23 experts at the municipal level have provided guidance off-site, including 8 experts at the municipal level, 4 of whom specialize in Western medicine and 4 who specialize in traditional Chinese medicine. The implementation of a multi-disciplinary comprehensive diagnosis and treatment mode with concentrated specialties and experts helps to provide quality medical care in an attempt to increase the cure rate and reduce the mortality rate.

ii) *Personalized Treatment Plans for Patients in Severe or Critically Ill Condition Patients.* For patients in severe or critically ill condition (*6*), a refined diagnosis and treatment mode − A Dedicated Team and a Personalized Treatment Plan – has been implemented. A high-level collection of specialists in infectious diseases, respiratory critical care, and critical care medicine holds consultations. Six of these specialists are resident experts who are on-call day and night. They are responsible for group rounds twice a day (once in the morning and once in the evening), and hold sequential consultations regarding all patients in severe condition. In addition, 5 critical care experts from the front line of intensive care medicine and respiratory critical care in municipal hospitals lead 5 medical teams in the intensive care unit. Each team is in charge of 2 patients in critical condition and 2 patients in severe condition. These teams are responsible for the clinical treatment of all critically ill patients, ensuring timely detection of changes in a patient's condition and adjustment of treatment strategies. Depending on the care needs of critically ill patients, a special treatment team for extracorporeal membrane oxygenation (ECMO) treatment, continuous renal replacement therapy (CRRT) treatment, respiratory therapy, psychotherapy, and other specialized treatments is stationed in the ward to specifically manage patients. The Shanghai COVID-19 Medical Treatment Expert Group has established an effective clinical treatment plan and a proven medical treatment management system for the treatment of patients with COVID-19. Based on the summaries of early clinical diagnosis and treatment, the Expert Group continues to use a combination of hormones, vitamin C, heparin, interferon (developed by the SPHCC), and thymus peptides to effectively inhibit the progression of severe cases. Once the unified treatment plan was adopted, several imported cases with risk factors for progression have been prevented from developing into severe disease. On the basis of this treatment plan, the transition from severe condition to critically ill condition is avoided through the use of high-flow oxygen, deep breathing, and other techniques.

iii) *Well-organized Classification of Patients.* Based on the principle of grading, the admission procedures in emergency wards have been devised scientifically, and the medical workers have been sensibly deployed in order to guarantee the timely treatment for patients. Due to the differences in how patients arrive at the Center, treatment protocols and personal protection procedures for outpatients and inpatients with an unidentified fever have been devised, including four main procedures for patient pre-examination and triage, laboratory testing, prevention of nosocomial infection, and patient transfer. All of these procedures help to improve the ability to admit patients and reduce the risk of nosocomial infection in an effective manner.

iv) *Establishment of Transitional Wards.* The Center had a maximum capacity of 250 infected patients. Faced with an overflow, a stratified triage strategy was promptly adopted. Four negative pressure isolation wards, A3, A4, A1, and A2, were successively created. For patients in severe condition, early identification and intervention is the key, and timely triaging of patients should be done depending on their disease status. Therefore, patients with severe COVID-19 were transferred to A3, and patients with mild COVID-19 were concentrated in A1, A4, and A2. In line with changes in the patient's condition, the infected are thus treated in the isolation ward, transition ward, and then the observation ward.

v) *Nosocomial Infection Prevention and Control.* The level of protection, personal protective equipment, and disinfection measures are clearly specified. The standards and procedures for donning and removing protective equipment have been upgraded, and training and evaluation have been enhanced. To reduce the risk of nosocomial infection, non-contact sensing devices are used to sterilize and transport goods. In dressing areas, a bi-directional voice and video surveillance system is used so that real-time guidance is available during the process of donning and removing protective equipment. Due to safety concerns, an aerosol monitoring system and a personnel and equipment sterilizer have been installed to adjust air purification efficiency. Reused items are highly disinfected by the Center's central sterile supply room while vehicles for patient transfer are thoroughly disinfected on the Center's premises. A quality medical waste incinerator is exclusively used to dispose of medical waste and medical waste is traceable, which helps to eliminate secondary contamination.

vi) *Identification and Reporting of the Asymptomatic.* There are four ways to identify the asymptomatic, that is, medical observation of close contacts, an investigation of an outbreak in clusters, tracing of the source of infection, and identification of people with a history of travel or residence in regions where COVID-19 is present. The monitoring of the asymptomatic is mainly reflected in the enhancement of targeted screening, where the scope of monitoring is

further expanded to active screening of close contacts of confirmed cases and asymptomatic patients, outbreaks in clusters, target areas, and groups. Communities and fever clinics play a key role in surveillance, where tracing the source of infection is assisted by clues to the infection. An epidemiological examination of the confirmed asymptomatic is conducted in a timely manner, and relevant information is openly available. The requirements for reporting, epidemiological examination, and management of close contacts of asymptomatic patients are basically the same as those for confirmed cases. Once an asymptomatic patient is identified by a medical facility at any level, a direct report must be submitted online within two hours and an epidemiological examination must be completed within 24 hours. The confirmed asymptomatic will be quarantined at a designated facility for 14 days for medical observation; if they test negative for a coronavirus twice, they can be released from quarantine.

SPHCC will accelerate the construction of the National Emergency Medical and Strategic Reserve Center for Public Health. This facility will have three core functions: providing medical treatment, conducting scientific research and improving technical expertise, and conducting external exchanges and training. During the COVID-19 pandemic, we are providing the highest level of emergency medical care and will continue to do so.

Funding: This work was supported by *i*) A research project of the Shanghai Health Committee (grant no. 201940014 for Strategic development of an infectious disease hospital based on dynamic capabilities theory, *ii*) A project of the Shanghai Shenkang Hospital Development Center to optimize clinical management (grant no. SHDC12019632 for The long-term operation of the Yangtze River Delta infectious disease prevention and control alliance based on the theory of strategic alliances, and *iii*) A research project of the Shanghai Health Committee (grant no. 202040046 for Optimization of the response to and care for emerging severe infectious diseases based on designated hospitals for treatment of infectious diseases).

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

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Received September 3, 2020; Revised January 21, 2021; Accepted January 28, 2021.

Released online in J-STAGE as advance publication February 7, 2021.

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Global Health & Medicine. 2021; 3(2):115-118.

LETTER

DOI: 10.35772/ghm.2020.01093

Japanese WHO Collaborating Centres (WHO CCs) fight against COVID-19

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Abstract: WHO Regional Office for the Western Pacific (WPRO) organized an online meeting connecting WHO Collaborating Centres (WHO CCs) in the region on 25 August 2020, to share experiences and promote networking on COVID-19 response. The meeting shared regional update on situation and responses, and COVID-19 related experiences of selected WHO CCs, followed by discussions on opportunities for enhancing collaboration between WPRO and WHO CCs. Priorities of WPRO's support to countries included a health systems approach rather than single intervention. On behalf of WHO CCs in Japan, the National Center for Global Health and Medicine (NCGM) delivered a presentation on the results of a survey about COVID-19 related activities of these WHO CCs. These activities were categorized into collaboration with WHO, research and development, public health responses, and clinical services. Collaboration with WHO included sending consultants through the scheme of GOARN, strengthening of COVID-19 testing, and contribution to development of WPRO guidelines. Research and development involved establishment of a nationwide registry of COVID-19 clinical data. Following the meeting, NCGM further enhanced its activities as WHO CC. Since WHO CCs in the country have a wide range of expertise that could contribute to health system strengthening, it is worthwhile for the WHO CCs to consider amending existing work plans for supporting countries in the region to incorporate a health systems approach as part of COVID-19 response strategies.

***Keywords*:** COVID-19, World Health Organization (WHO), Collaborating Centre (CC), Western Pacific Region (WPRO)

World Health Organization Collaborating Centres (WHO CCs) are instrumental partners that provide strategic support for implementing WHO's mandate and programmes and in developing and strengthening institutional capacity. Exchange of information and experiences among WHO CCs in Japan has been promoted through networking meetings hosted by the National Center for Global Health and Medicine (NCGM) since 2017. WHO Regional Office for the Western Pacific (WPRO) organized an online meeting connecting WHO CCs in the Western Pacific Region (WPR) amid the COVID-19 response on 25 August 2020. This meeting aimed to share experiences of WHO CCs' response and promote networking on COVID-19 response and towards the "new normal". It was attended by around 250 participants from 10 countries.

At the beginning of the meeting, Dr. Takeshi Kasai, the Regional Director, introduced the overview of the WPRO's new vision paper titled "For the Future" published in January 2020 (*1*). He expressed his strong expectations that WHO CCs urgently tackle issues related to vulnerable health and social systems, which have surfaced due to the COVID-19 pandemic. It was followed by presentations on regional epidemiological trends and a programmatic update on COVID-19 as well as the online survey on WHO CCs' response to COVID-19. The survey results indicated more than 80% expressed their interest in collaborating to support the COVID-19 response in the region while fewer than 50% were actually working with WHO for that purpose. WPRO requested WHO CCs to consider possible support for WPRO's COVID-19 response structure, discuss possible support with WPRO's focal points for respective WHO CCs, and amend the work plan of each WHO CC as needed. Four WHO CCs in Australia, China, Japan, and Republic of Korea then reported their activities, challenges, and opportunities in responding to COVID-19 in each country. The National Center

Figure 1. The presentation by NCGM President Norihiro Kokudo at the online meeting connecting WHO CCs in the WPR amid the COVID-19 response on 25 August 2020. NCGM: National Center for Global Health and Medicine; WHO CCs: WHO Collaborating Centres; WPR: Western Pacific Region.

for Global Health and Medicine (NCGM) delivered a presentation on behalf of WHO CCs in Japan (Figure 1).

Prior to this meeting, NCGM conducted an additional survey to collect detailed information on COVID-19 related activities of WHO CCs in Japan. Out of the 37 WHO CCs (including one CC in re-designation process), 24 CCs responded and 21 CCs indicated they had ongoing or planned activities related to COVID-19. These activities were categorized into the following four domains, and the brief summary of each domain in Table 1 was presented by NCGM during the meeting.

i) *Collaboration with WHO* A WHO CC coordinated the recruitment of consultants, who worked for the WPRO and WHO country office in the Philippines through the scheme of Global Outbreak Alert and Response Network

Table 1. Main activities by WHO Collaborating Centres (WHO CCs) in Japan

Items	Activities
i) Collaboration with WHO	● Sent technical consultants to Global Outbreak Alert and Response Network (GOARN) ● Contributed to Webinars by WHO/WPRO ● Provided technical support for handling PCR testing to seven countries ● Technical support to WPRO Guidance on COVID-19 for the care of older people and people living in long-term care facilities, other non-acute care facilities and home care ● Translated WHO materials into Japanese √ Water, Sanitation and Hygiene (WASH) COVID-19 √ Use of Chest Imaging in COVID-19; A Rapid Advance Guide √ Mental health documents related to COVID-19 ● Staff members joined WHO movie material
ii) Research and Development	● Established registry of COVID-19 cases all over Japan (6,003 cases/525 institutions registered) ● Promoted and conducting clinical trials and various studies on medical treatment ● Study on effective border control including SARS-CoV-2 testing at the points of entry ● Development of √ vaccine √ testing for SARS-CoV-2 antigen √ test kits (dry LAMP) to differentiate SARS-CoV-2 and Influenza (A&B) √ anti-viral drug herbal medicine ● Survey of sewage and raw water ● Planned surveys on √ people's behaviors such as dietary life and physical activities √ impact of COVID-19 on TB programs in several countries
iii) Public Health Response	● Advice and contribution to national government's COVID-19 responses √ Technical support to the government as leading national institutions √ Outbreak containment operations in the outbreaks on cruise ship √ Screening of COVID-19 infection among returnees on charter flights from Wuhan, China √ Managing temporary accommodation using hotels for isolating positive cases √ Development of national guidelines of COVID-19 ● Advice and contribution to Tokyo Metropolitan's COVID-19 responses √ Advice on infection prevention and control √ Management of temporary facility for isolating mild or asymptomatic COVID-19 cases ● Conducted surveillance for COVID-19 and co-infection of COVID-19 and Influenza ● Preparation for upgrading quality control system for vaccine importation ● Expanded capacity of PCR testing ● Supported Ministries to issue circulars on swimming pool, water purification and sewage system
iv) Clinical Services	● Disseminated updated information and provided technical advice on diagnosis, treatment, and infection prevention and control to medical facilities nationwide ● Established and coordinated a novel clinical network for early case detection and case management ('Shinjuku Model') ● Provided medical services for COVID-19 cases √ Severe cases using respirators and ECMOs √ Adults and children ● Online management of mild cases

(GOARN) (*2*). They provided technical assistance on COVID-19 including strengthening of infection prevention and control, staff training, and surveillance. A WHO CC provided technical support for handling Polymerase Chain Reaction (PCR) testing to seven countries including countries in WPR such as Mongolia and Viet Nam. Technical inputs were also provided for developing WPRO guidelines such as 'Guidance for the care of older people and people living in long-term care facilities, other non-acute care facilities and home care'. Moreover, several WHO materials were translated into Japanese. They included COVID-19 documents concerning water, sanitation, and hygiene (WASH), chest imaging, and mental health.

ii) *Research and Development* A wide range of research and development was underway. Clinical trials on medical treatment, as well as studies on vaccine, testing and medicines have been accelerated (*3*). A nationwide registry of COVID-19 clinical data was established and 6,003 cases were registered from 525 institutions as of August 2020. It aims to reveal clinical epidemiological characteristics of inpatients in Japan, including comorbidities, progression to severe cases and trend of mortality (*4*). A study was conducted on epidemiology and quarantine measures (*5*). Ongoing or planned studies include surveys to examine COVID-19 in sewage and raw water, and to assess the impact of COVID-19 on national tuberculosis programs in low and middle countries, and on people's dietary and physical behaviors.

iii) *Public Health Response* Many WHO CCs have been very active in contributing to the public health response to COVID-19 in Japan. As leading national institutions, several WHO CCs played a central role in advising national and Tokyo metropolitan governments on various technical matters such as outbreak containment operations, infection prevention and control, and surveillance. WHO CCs also contributed to the development of national guidelines and ministerial circulars, and capacity-building of PCR testing.

iv) *Clinical Services* As top referral hospitals, WHO CCs disseminated updated information and provided technical advice on diagnosis, treatment, and infection prevention and control to medical facilities with a view to building the capacity of medical facilities throughout the country for managing COVID-19 cases. It is noteworthy that a novel clinical network model was developed across the continuum of COVID-19 testing, temporary accommodation for asymptomatic or mild cases, and referral of moderate and severe cases to hospitals. Several WHO CCs accumulated extensive experiences of treating and managing very severe COVID-19 cases using respirators and extracorporeal membrane oxygenation (ECMOs).

Moreover, in collaboration with WHO headquarters, WPRO, GOARN and the Japanese research group on human resource development for international outbreak responses, NCGM as WHO CC organized the first virtual GOARN Tier 1.5 workshop on infection prevention and control (IPC) targeting IPC experts in Japan on 29-30 October 2020. NCGM coordinated the dispatch of a Japanese clinical expert through GOARN for supporting COVID-19 response in Papua New Guinea. Upon request of WPRO, a series of seminars on COVID-19 clinical management were held for clinicians in the region. Further, a training module on preventing and managing COVID-19 cases in medical facilities was developed by NCGM, which will be used in a training course for one of the largest hospitals in Vietnam in early 2021. Regarding research in Japan, the nationwide registry of COVID-19 clinical data was expanded to reach 17,197 cases from 830 institutions as of November 2020. In the area of public health response, NCGM launched an initiative to improve information flow and access to health services, particularly for Vietnamese, Myanmar and Nepalese communities to prevent and address the expansion of COVID-19 transmissions among foreign residents in Japan (*6*). As for clinical services, NCGM as one of four nationally designated hospitals served 389 COVID-19 patients including very severe cases, as of 12 December 2020 (*7*).

Regarding the way forward, since the pandemic will likely continue for the next few years, it is critical to establish a sustainable response to COVID-19. Priorities of WPRO's support include: *i*) early detection and targeted response; *ii*) expanding "social capacity" (*e.g.* public heath, health system, protection of vulnerable population); *iii*) voluntary behavioral changes at individual and organization-level; *iv*) "Backcasting" from future needs; and *v*) a health systems approach, rather than single intervention.

WHO CCs in Japan have been working with WPRO and countries in the region to enhance response to COVID-19. For example, specific technical support has been provided through GOARN and other WHO schemes. Ongoing nationwide registry of COVID-19 could inform global and regional clinical guidelines. Furthermore, these and other WHO CCs in the country have a wide range of expertise that could contribute to health system strengthening, such as health workforce, nursing, service quality, mental health, laboratory, as well as disaster preparedness, response and recovery. In line with WPRO's priorities, it is worthwhile for the WHO CCs to consider amending existing work plans for supporting countries in the region to incorporate a health systems approach as part of COVID-19 response strategies.

Acknowledgements

The authors thank Dr. Takeshi Kasai, and other staff of WHO Regional Office for the Western Pacific. The authors' appreciation goes to all WHO CCs

listed in the supplementary Table S1 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=12*).

Funding: None.

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

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Received October 22, 2020; Revised January 27, 2021; Accepted February 4, 2021.

Released online in J-STAGE as advance publication February 11, 2021.

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Global Health & Medicine. 2021; 3(2):119-121.
LETTER
DOI: 10.35772/ghm.2020.01081

Care for children's mental health during the COVID-19 pandemic in Japan

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Abstract: COVID-19 causes very serious issues all over the world. In Japan, the number of new infections in Tokyo exceeded 2,000 for the first time on 7 January 2021, and the situation is becoming increasingly serious. Japan is in the midst of its third big outbreak. Japanese society will face several challenges regarding children's mental health during the COVID-19 pandemic. In order to develop healthy minds in children, it is important to view the changes in children's minds in a positive light and promote their healthy emotional development while correctly fearing COVID-19. This sense of social stagnation and uncertainty is likely to increase feelings of insecurity and isolation among children. It is also important to prevent the repetition of child abuse in the home due to parental unemployment, alcohol problems, and reduced contact with non-family members in stay home and the recession as a result of COVID-19. During the pandemic, adults should be sensitive to the unusual behavior of children. We propose six suggestions of care for children during the COVID-19 pandemic.

***Keywords*:** child, mental health, COVID-19, pandemic, school closure

COVID-19 causes very serious issues all over the world. In Japan, the number of new infections in Tokyo exceeded 2,000 for the first time on 7 January 2021, and the situation is becoming increasingly serious. Japan is in the midst of its third big outbreak. In many countries, children's mental health during the COVID-19 pandemic is a growing problem (*1,2*). COVID-19 has caused unprecedented challenges at all levels of society. Therefore, Japanese society will face several challenges regarding children's mental health during the COVID-19 pandemic.

Children's daily activity during COVID-19 pandemic in Japan. There were significant changes in children's mental health during the first outbreak. Children's daily life was changed after Japanese Prime Minister ordered all elementary and junior high schools in Japan to close their schools temporarily from 2 March 2020 to spring break in response to the explosive spread of the disease in Japan. After schools were reopened in June, the school day was dispersed. It seemed as if the spread of the infection was under control, but then COVID-19 once again raged, and the number of infections across the country began to increase. In the summer, the classroom windows are open while class is in session. If there is a cluster outbreak in a school, the school is closed for a few days of disinfection. School travels and sports events had been cancelled, and the regular school curriculum has not been implemented.

Children's mental health during COVID-19 pandemic in Japan. Every child lives and grows up in a group at home and at school. The need for peer groups is especially high during adolescence. Adolescents grow up independent and prioritize peer connections over their parents, which can pose significant challenges to young people's healthy emotional development if it is stifled. Adolescents may grieve the rites of passage they were supposed to experience and may feel anxious about an uncertain future in the face of COVID-19. With the spread of COVID-19, many children have had a difficult time participating in population activities. In order to develop healthy minds in children, it is important to view the changes in children's minds in a positive light and promote their healthy emotional development while correctly fearing COVID-19. This sense of social stagnation and uncertainty is likely to increase feelings of insecurity and isolation among children. It is also important to prevent the repetition of child abuse in the home due to parental unemployment,

alcohol problems, and reduced contact with non-family members in stay home and the recession as a result of COVID-19 (Figure 1).

COVID-19 pandemic effects not only directly affect the child, but also greatly affect the environment surrounding the child, which ultimately affects the child; measures being taken to control the spread of COVID-19 put the child at a higher risk of neglect and abuse, and put the child at a higher risk for neglect and abuse than COVID-19 as a "second wave" (*3*). Furthermore, some children and adults were enthusiastic about game devices and they could not buy game devices before COVID-19 pandemic (*4-6*). Stay home caused these problems.

Child and adolescent psychiatry during COVID-19 pandemic in Japan. During COVID-19 pandemic, there are unprecedented symptoms from childhood to adulthood (*1*). Some children have noted some psychiatric symptoms during pandemic and will often lead to early visits to child psychiatrists and pediatricians (*1,7*). Figure 2 shows the number of outpatients who visited the Department of Child and Adolescent Psychiatry, Kohnodai Hospital, National Center for Global Health and Medicine in 2020 compare with 2019. The number of outpatients in follow-up examinations is decreasing. However, the number of outpatients in the first visit increased after 1 April . Especially, the number of children with anorexia nervosa has had a large increase of 2.2 times from 2019 to 2020 in our hospital, which is the same as other counties (*8*). In a questionnaire to the parents of outpatients, 63 (44%) of 143 responses postponed their visit to the hospital (*7*). The reasons for this postponement were infection control not only for the children and their parents but also for the grandparents living with them, not having child issues, but still having medicine left.

Care for children's Mental health during COVID-19

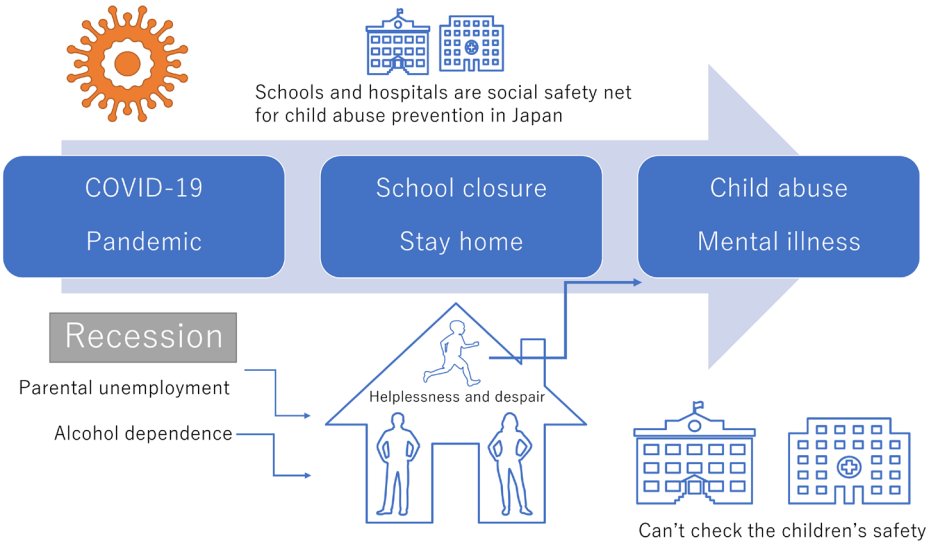

Figure 1. Child abuse under COVID-19 pandemic. Measures being taken to control the spread of COVID-19 put the child at a higher risk of neglect and abuse.

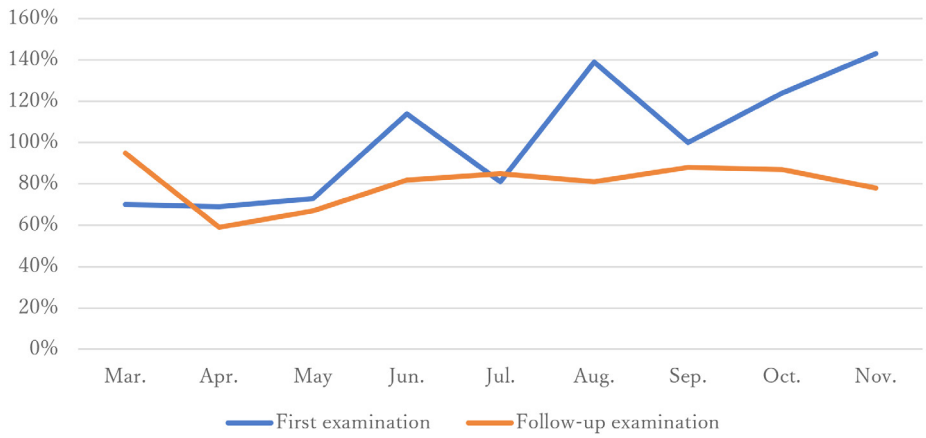

Figure 2. The number of outpatients of first and follow-up examination in 2020 compared with 2019.

Table 1. Six suggestions of care for children during COVID-19 pandemic

No.	Suggestions
1	Some children and parents misunderstood knowledge about COVID-19. The correct information can help reduce undue anxiety.
2	It is a natural reaction to children's anxiety in pandemic. In addition children usually are not able to be aware of this change on their own. Children are characterized by a tendency for changes in feelings to lead to physical symptoms.
3	Discuss changes in the child's mood and behavior, changes in appetite, sleep and other aspects of life. Clinicians should talk about the changes and concerns without rushing to resolve them.
4	Look for children's positive changes and positive changes as a family.
5	Solution for problem of Internet gaming need strong relationships between parents and children to discuss and work together to address problems. The discussion about the problem causes rebellion and resistance from the child. It is normal for there to be a gap between the attitudes of the child and family to solve. The relationship between parent and child on a daily basis is important.
6	Key points of communication with the child: *i*) Do not blame the child. *ii*) Discuss the rules with respect to the child's will and opinion. *iii*) Praise the child.

pandemic in Japan. During pandemic, adults should be sensitive to the unusual behavior of children. Children who are less likely to be infected and less likely to become seriously ill are protecting their stay homes, washing their hands and wearing masks to protect adults who are more likely to have severe illness. Children who are less likely to be infected and less likely to have a serious state, masks to protect adults who are more likely to have a severe state. Adults should remember to appreciate their children.

In conclusion, the first step is to take an interest in your child about how they understand COVID-19. With so much information coming from the internet and social networking sites, it is not uncommon for children to be misinformed and overly anxious. It is important to discuss COVID-19 with children, whether you are a parent, teacher or physician. As summarized in Table 1, we propose six suggestions of care for children during COVID-19 pandemic.

Funding: None.

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

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Received September 9, 2020; Revised February 12, 2021; Accepted February 22, 2021.

Released online in J-STAGE as advance publication March 6, 2021.

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Global Health & Medicine. 2021; 3(2):122-124.
LETTER
DOI: 10.35772/ghm.2020.01119

The effect of the COVID-19 pandemic on incidence and characteristics of pulmonary embolism

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Abstract: The coronavirus disease 2019 (COVID-19) pandemic has affected presentations of conditions unrelated to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection itself. We investigated the pandemic's effect on incidence and characteristics of pulmonary embolism (PE) cases without the infection. We retrospectively compared non-COVID PE patients during January 16-August 31, 2020 (COVID period) with PE patients during the same period in 2017-2019 (Pre-COVID period). The number of out-of-hospital onset cases was significantly higher during the pandemic than during each of the pre-COVID years. Also, the patients in the COVID period were older, more likely to be free of thrombotic predispositions, had higher mortality risks of PE, and were more likely to arrive at the hospital on emergency transport. Sedentary lifestyles during the pandemic seem to have had considerable effects on presentations of PE.

***Keywords*:** COVID-19, venous thromboembolism, SARS-CoV-2, quarantine

The ongoing 2019 coronavirus disease (COVID-19) pandemic has altered people's habits and attitudes toward healthcare, causing a decreased use of health services for non-COVID-19 conditions (*1*). Pulmonary embolism (PE) is a common complication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (*2*). However, few studies have analyzed PE in patients without COVID-19 during the pandemic. As people adopt more sedentary lifestyles due to stay-at-home restrictions, PE prevention is becoming more important. We investigated the effect of the COVID-19 pandemic on the incidence and clinical characteristics of PE in patients without COVID-19 in Tokyo, Japan.

We retrospectively examined adult patients with imaging-confirmed PE without COVID-19 treated at our tertiary-care hospital during the "COVID period" (January 16 to August 31, 2020) and the control "Pre-COVID period" (January 16 to August 31, 2017-2019). Patients with SARS-CoV-2 infection were excluded. Eligible patients were grouped according to onset and treatment: patients who received inpatient or outpatient treatment, and patients with in-hospital onset (*i.e.*, hospital-acquired) PE. For cases with out-of-hospital onset, we collected data on patient demographics (age, sex, and body mass index) and PE risk factors according to previous literature (*3*). To analyze PE severity, we calculated early mortality risk categories per European Society of Cardiology (ESC) guidelines (*3*) and also noted if emergency medical services (EMS) were used. All data were collected from electronic medical records. Continuous and ordinal variables were compared using the Mann-Whitney *U* test, and nominal variables were compared using Pearson's chi-squared test. The study protocol was approved by the ethics committee of the National Center for Global Health and Medicine.

We identified 152 patients with PE during the control period and 77 during the COVID period, including 2 patients with positive SARS-CoV-2 tests that were excluded (from the analyses). The number of patients with non-COVID PE during the COVID period was significantly higher than during each of the pre-COVID years, with the highest increase in patients with non-hospital-acquired PE (Table 1a). Patients with non-hospital-acquired PE during the COVID period were older ($p = 0.013$), more likely to be free of identifiable thrombotic predispositions ($p = 0.012$), had a significantly higher ESC early mortality risk ($p < 0.001$), and were more likely to be transported to the hospital by EMS ($p = 0.019$) than patients during the pre-COVID control period (Table 1b).

PE in the COVID-19 era has been greatly affected by the pandemic. The fact that PE patients in the COVID period were more likely to be free of identifiable thrombotic predispositions suggests that lifestyle effects of the pandemic played a key role in the increase of non-hospital-acquired PE. The non-compulsory stay-at-home request in Tokyo was considered milder than that in other countries. Nonetheless, large-scale data collected from mobile devices indicate a reduction in mobility for an

Table 1a. Trend in all PE incidences during pre-COVID and COVID periods

Variables	Pre-COVID period			COVID period
	2017	2018	2019	2020
Total	50	44	58	75
Setting at onset				
Out-of-hospital onset	19	17	20	36
Outpatient treatment	7	12	11	12
Inpatient treatment	12	5	9	24
In-hospital onset	31	27	38	39

Table 1b. Comparison of out-of-hospital onset PE during pre-COVID and COVID periods

Variables	Pre-COVID period	COVID period	*p* value
Total	56	36	
Patient demographics			
Age, years	65 [47.5-72]	74 [58-79.25]	0.013
Male sex, *n* (%)	30 (54%)	18 (50%)	0.74
BMI, kg/m^2	22.13 [15.3-25.41]	22.04 [19.99-24.24]	0.55
Any thrombotic medical condition[§], *n* (%)	43 (77%)	23 (64%)	
Cancer, *n* (%)	26 (46%)	11 (31%)	
Diabetes mellitus, *n* (%)	4 (7%)	7 (19%)	
Obesity, *n* (%)	6 (11%)	0 (0%)	
Congestive heart failure or chronic respiratory disease, *n* (%)	6 (11%)	6 (17%)	
Orthopedic condition[‡], *n* (%)	7 (13%)	6 (17%)	
Oral contraception or hormone therapy, *n* (%)	3 (5%)	3 (8%)	
Recent long travel, *n* (%)	7 (13%)	0 (0%)	
None of the above risk factors, *n* (%)	2 (4%)	7 (19%)	0.012
PE severity			
ESC early mortality risk			< 0.001
Low risk, *n* (%)	42 (75%)	14 (39%)	
Intermediate-low risk, *n* (%)	11 (20%)	13 (36%)	
Intermediate-high risk, *n* (%)	1 (2%)	6 (17%)	
High risk, *n* (%)	2 (4%)	3 (8%)	
Use of EMS, *n* (%)	12 (21%)	16 (44%)	0.019

[§]Thrombotic medical conditions: autoimmune diseases, cancer, chronic respiratory disease, congestive heart failure, diabetes mellitus, infection, inflammatory bowel disease, obesity, paralytic stroke, previous venous thromboembolism, thrombophilia, varicose veins. [‡]Orthopedic conditions: fracture of lower limb, hip or knee replacement, major trauma, spinal cord injury. Abbreviations: BMI, body mass index; ESC, European Society of Cardiology; EMS, emergency medical service. Data are expressed as number (%) or median [interquartile range].

extended time following the first wave of the pandemic (*4*). Limited ambulation is the most common risk factor of PE and is present in 45% of patients with venous thromboembolism (VTE) (*5*), and sedentary lifestyles are widely recognized to be closely associated with VTE (*6*). This study demonstrates that even lenient approaches to contain the pandemic impact other health domains.

Previous studies have reported that the elderly population exhibited the most significant decrease in physical activity due to self-quarantines (*7,8*). Staying indoors is generally accepted as essential for the elderly population, who are more susceptible to COVID-19. Quarantines are especially reinforced in nursing homes. However, it is important to also consider that older adults, even without comorbidities, carry a higher risk of developing severe PE. The public must be made aware of the risks of sedentary behavior during the pandemic.

This study is limited by its retrospective approach and inclusion of patients from a single institution. Multi-center studies are needed to confirm if this is a national or global trend.

Funding: This work was supported in part by Grants-in-Aid for Research from the National Center for Global Health and Medicine (20A-3002).

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

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Received December 30, 2020; Revised January 25, 2021; Accepted February 4, 2021.

Released online in J-STAGE as advance publication February 15, 2021.

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Print ISSN: 2434-9186 Online ISSN: 2434-9194

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Global Health & Medicine

Volume 1, Number 1
October, 2019

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