Had COVID-19 spread in the community before the first confirmed case in Nagasaki, Japan?

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Abstract

This retrospective study evaluated stored nasopharyngeal swab samples from Japanese patients with influenza-like illness during the 2019/2020 season. We aimed to determine whether COVID-19 had spread in the community before the first confirmed case. The period of influenza season during 2019/2020 in Nagasaki was shorter than in previous influenza seasons. When the first COVID-19 case was reported in Nagasaki prefecture, the number of influenza cases were very low. No positive results for SARS-CoV-2 were detected in 182 samples that were obtained from adult outpatients. Our results revealed no large-scale spread of COVID-19 in the community before the first confirmed case.

1. Introduction

The first outbreak of coronavirus disease-19 (COVID-19) in Wuhan, China was reported on December 31, 2019. However, symptom onset in the first COVID-19 case from the first outbreak occurred on December 8, 2019 [1], and there is a possibility that COVID-19 had spread worldwide before the first reported outbreak. In France, the COVID-19 epidemic began during late January 2020, although Deslandes et al. have reported a patient with a stored respiratory sample from late December 2019 that was positive for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [2]. In Japan, the first confirmed COVID-19 case was reported on January 14, 2020, although the patient had returned from China on January 6, 2020. Thus, testing for SRAS-CoV-2 has been conducted for individuals who return from COVID-19 endemic areas. The first confirmed COVID-19 case in Nagasaki prefecture involved a man who had close contact with a COVID-19 case in another area, and SARS-CoV-2 was detected on March 14, 2020. However, there is a possibility that COVID-19 had spread in the community before that date, as Nagasaki prefecture is geographically close to China and had accepted many Chinese tourists, including one cruise ships. Furthermore, the number of patients with influenza was lower than normal during the 2019/2020 season. As the clinical symptoms of COVID-19 and influenza are similar, we conducted a retrospective study of stored nasopharyngeal swab samples from patients in Nagasaki with influenza-like illness during the 2019/2020 season. These specimens had been collected as part of a previous study regarding

novel influenza tests [3], and were used in the present study to evaluate whether COVID-19 had spread in the community before the first confirmed case.

2. Materials and Methods

2.1. Ethics

This retrospective study was approved by the ethics committee of Nagasaki University Hospital (20061508) and was registered in the UMIN Clinical Trials Registry (UMIN000040278). The patients had previously provided informed consent for sample collection and data publication as part of a previous study regarding novel influenza tests [3].

2.2. Data and sample collection

A number of weekly influenza cases per sentinel hospital or clinic in Nagasaki prefecture between the 2015/2016 and 2019/2020 seasons were obtained from a weekly influenza report provided by the National Institute of Infectious Diseases [4]. Influenza season was defined as when a number of weekly influenza cases per sentinel hospital or clinic was 1.0 or more. Data regarding newly confirmed cases in Nagasaki prefecture were obtained from a prompt report provided by the Nagasaki Prefectural Government [5]. Patient characteristics were collected from the previous study's database [3]. The nasopharyngeal swab samples had been collected between November 15, 2019 and March 14, 2020 from 182 adult outpatients with influenza-

like illness who had one or more symptoms, such as fever (≥38°C or an increase of ≥1°C from normal body temperature), nasal discharge, nasal congestion, sore throat, cough, headache, chills, fatigue, joint pain, or muscle pain at 4 hospitals and clinics in Nagasaki prefecture [3]. Patients treated with anti-influenza agents 1 month prior to the clinic or hospital visit were excluded in the previous study [3]. Two sets of nasopharyngeal swab samples were collected from each patient. One set was immediately subjected to automated immunochromatographic antigen tests for detecting influenza virus (BD Veritor System Flu; Becton, Dickinson and Company, Tokyo, Japan) and the other set was stored in 3 mL of universal transport medium (Copan Italia spa, Brescia, Italy) at –80°C until further analysis [3].

2.3. Detection of SARS-CoV-2

Nucleic acid amplification assays were performed using a protocol that was published by the National Institute of Infectious Diseases [6]. Total nucleic acids were extracted from the samples using the MagMAX Viral/Pathogen Nucleic Acid Isolation kit (ThermoFisher Scientific) according to the manufacturer's protocol. The detection of SARS-CoV-2 RNA was performed using 5 μL of RNA template, real-time reverse transcription (RT)-PCR primers (forward: AAATTTTGGGGACCAGGAAC, reverse: TGGCAGCTGTGTAGGTCAAC), and the 2019-nCoV_N2 probe (5'-FAM-ATGTCGCGCATTGGCATGGA-BHQ1-3'). The PCR was performed using the Thunderbird probe one-step qRT-PCR Kit (TOYOBO) and a Rotor-

Gene Q 5plex HRM System (QIAGEN). Viral copies were quantified using a standard curve from a 10-fold serial dilution of RNA transcripts, which was provided by the National Institute of Infectious Diseases. ~~Viral load was expressed as copies/5 μL.~~

3. Results

The influenza season during 2019/2020 in Nagasaki prefecture started in week 50 of 2019 and ended in week 6 of 2020 (Figure 1). This period was shorter than in previous influenza seasons. In addition, the peak weekly number of influenza cases per sentinel hospital or clinic was lower during 2019/2020 than during the previous five seasons. When the first COVID-19 case was reported in Nagasaki prefecture, the weekly case count was 0.6 influenza cases per sentinel hospital or clinic. The positive rates for influenza were 46.7% of cases during the pre-influenza period (weeks 46–49 of 2019), 45.0% during the influenza season (week 50 of 2019 to week 6 of 2020), and 22.2% during the post-influenza period (weeks 7–11 of 2020) (Figure 1). All 182 stored samples were subjected to RT-PCR to detect SARS-CoV-2, although none of the samples were positive for SARS-CoV-2.

4. Discussion

The duration of the influenza season in Nagasaki prefecture during 2019/2020 was shorter and the peak was lower than in previous seasons. Sakamoto et al. have also reported that the seasonal influenza activity was lower during 2020 than during previous years in Japan [7]. Furthermore, they reported that the number of influenza cases decreased from week 5, which is similar to our results. Thus, similar trends regarding influenza activity were observed in Nagasaki prefecture and Japan as a whole during 2020.

However, the first influenza case was confirmed in Nagasaki prefecture 9 weeks later than the first case in Japan (week 12 in Nagasaki and week 3 in Japan) [7]. In addition, Nagasaki prefecture is one of the Japanese regions that is geographically closest to China, with many Chinese cruise ships docking at the Nagasaki port until week 6 of 2020 [8]. These facts suggest that COVID-19 spread in the Nagasaki community might have influenced the low influenza activity, although we did not identify any positive results for SARS-CoV-2 in the nasopharyngeal swabs from 182 adult outpatients. Similarly, no positive results for SARS-CoV-2 were detected in studies of pre-pandemic nasopharyngeal swab samples from patients in Italy [9] and Switzerland [10]. Furthermore, nasopharyngeal swabs are the standard specimen for detecting SARS-CoV-2 [11], and the swabs for the present study had been obtained from healthcare workers and carefully stored [3]. Therefore, this study did not reveal evidence of COVID-19 spread in the Nagasaki community before the first confirmed case.

The present study is unable to clarify the reason for relatively low influenza activity during the 2019/2020 season in Nagasaki. In this context, Machida et al. have reported that implementation of infection prevention measures in the general population was insufficient at week 9 of 2020 [12]. However, Sakamoto et al. have suggested that concerns regarding COVID-19 may have influenced the detection of influenza, because of changes in symptomatic patients seeking medical attention or physicians choosing to test for influenza [7]. Thus, further studies are needed to clarify the reason for the low influenza activity during 2019/2020, and

whether it was related to concerns regarding COVID-19.

This study has several limitations. First, only a small number of samples were analyzed, and the participating centers were located in the southern district of Nagasaki prefecture. Thus, the results may not reflect the spread of COVID-19 throughout Nagasaki prefecture. Second, the present study analyzed stored samples and there is a possibility that negative results might be returned in cases with a low viral load, even if the samples were stored carefully. Third, the samples were obtained from outpatients and the results may be different among severely ill patients, as Deslandes et al. [2] detected SARS-CoV-2 in samples from patients who were treated in an intensive care unit.

In conclusion, our results revealed no large-scale spread of COVID-19 in the Nagasaki community before the first confirmed case.

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Conflict of Interest: The authors have no conflict of interest directly relevant to the content of this article.

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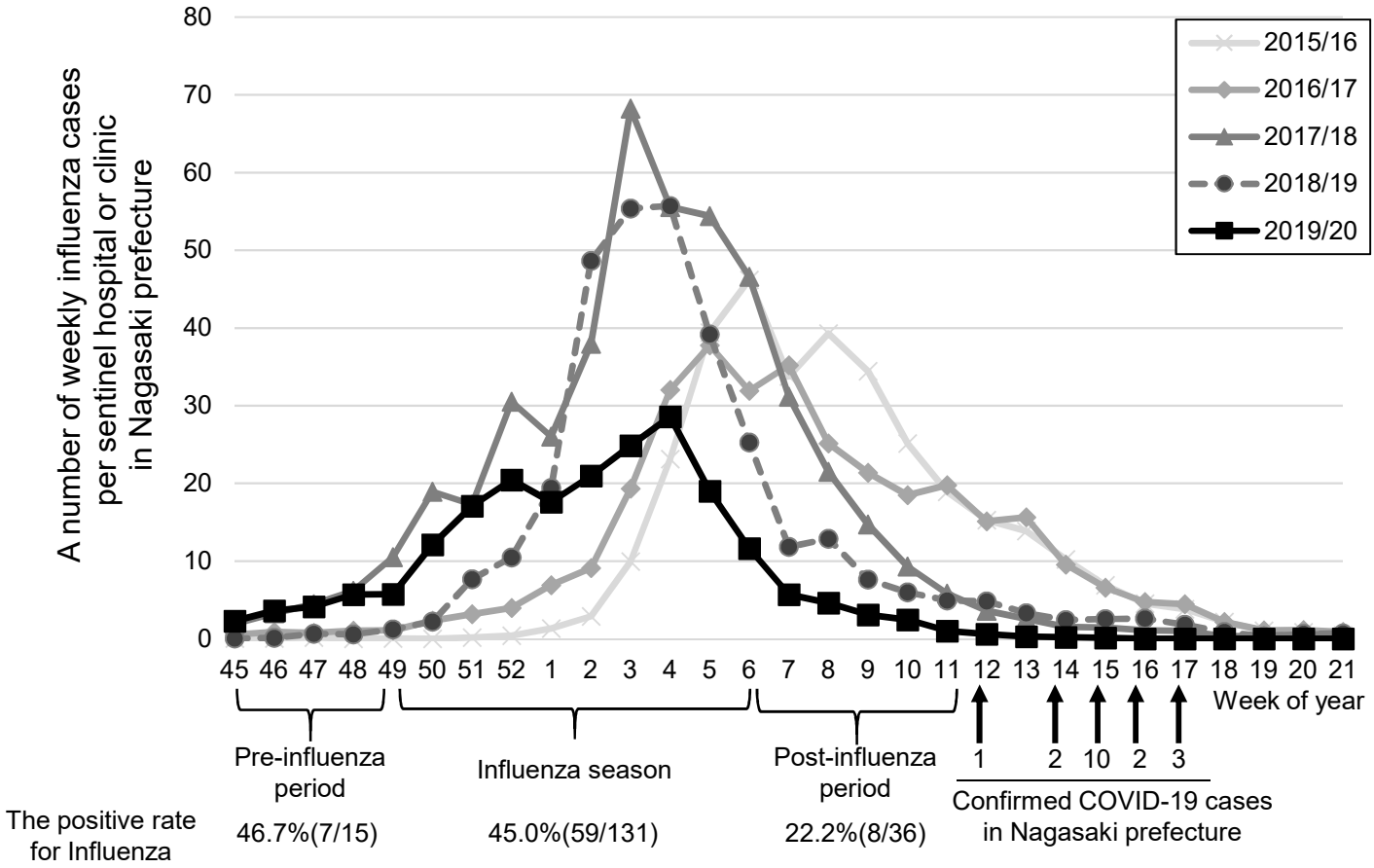
Figure legend

Figure 1. Influenza activity and confirmed COVID-19 cases in Nagasaki prefecture.



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