Large-scale serosurveillance of COVID-19 in Japan: Acquisition of neutralizing antibodies for Delta but not for Omicron and requirement of booster vaccination to overcome the…

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(Degree)
博士（医学）

(Date of Degree)
2022-03-25

(Resource Type)
doctoral thesis

(Report Number)
甲第8403号

(URL)
https://hdl.handle.net/20.500.14094/0100477829

（課程博士関係）

学位論文の内容要旨

Large-scale serosurveillance of COVID-19 in Japan: Acquisition of neutralizing antibodies for Delta but not for Omicron and requirement of booster vaccination to overcome the Omicron's outbreak.

日本での COVID-19 大規模血清疫学調査：デルタ株に対する中和抗体は得られているがオミクロン株に対しては得られておらず、オミクロン株の流行抑制には３回目のワクチン接種が必要である。

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1. Introduction

Several turning points have occurred since the COVID-19 pandemic's emergence in December 2019, and the pandemic has undergone drastic changes with its progress. One of the most important factors is the appearance of the continuous SARS-CoV-2 variants replacing the original variant. In Japan, the Alpha variant (B.1.1.7) replaced the existing strain by around April 2021; the Delta variant (B.1.617.2) then began spreading rapidly throughout the country from July to August 2021. The Omicron variant (B.1.1.529) was first reported in South Africa in November 2021 and caused largest increase of COVID-19 cases in Japan as of February 2022.

The other critical event in the pandemic has been the launch of COVID-19 vaccines. The mRNA vaccine was approved first in Japan and administered to healthcare workers starting in February 2021, then to individual aged ≥65 old beginning in April 2021, expanding to other populations from May 2021. The number of vaccinated individuals in Japan is increasing rapidly and reached approx. 74% of the population as of December 2021. A three-dose vaccination policy for medical staff was initiated in December 2021, and optional booster vaccination for all adults (≥18 years old) who got the 2nd vaccination over 8 months before has been started in Japan.

Periodic seroepidemiologic surveillance is useful to determine the precise COVID-19 situation. Our previous seroepidemiologic surveillance conducted at Hyogo prefecture in October 2020 revealed that 0.15% of 10,377 sera had neutralizing activity against SARS-CoV-2 infection.

In this study, we performed a follow-up seroepidemiologic surveillance in August and December 2021, which are the critical timings before and after the Delta variant outbreak in Japan, respectively.

2. Materials and Methods

We collected blood samples from a total of 2,000 individuals (1,000 each) who underwent a regular occupational health check-up at the clinics of Hyogo Prefecture Health Promotion Association in August 2021 and December 2021. Serum samples were subjected to the measurements of anti-N antibody with ECLIA (cobas e801 module, Roche Diagnostics), anti-S antibody with anti-S ELISA, and the neutralizing activity against different SARS-CoV-2 variants D614G (the original variant spread in Japan), Delta, and Omicron with live-virus neutralizing assay.

3. Results and Discussion

Large-scale seroepidemiologic surveillance of the anti-N positive rate

The SARS-CoV-2 anti-N antibodies which show past infection by SARS-CoV-2 were detected by the ECLIA, and 21 of the 1,000 samples (2.1%) in the August 2021 cohort were deemed positive, whereas 39 of the 1,000 samples (3.9%) in the December 2021 cohort were positive. In the August 2021 cohort, the anti-N positive rate for the age groups 30–39 and 40–49 were relatively high at 3.4% (6/179) and 4.1% (10/243), and no positive cases were observed for the age groups 18–19 and 70–83 years. The December 2021 cohort exhibited the noticeable positive rate 10.6% (11/104) for the age group 20–

29, and the difference was significant (P-value = 0.0014, Fisher's exact test). In contrast to the August 2021 cohort, the oldest age group (i.e., 70–79 yrs) of the December 2021 cohort showed the positive rate 5.0% (2/40), however the difference was not significant (P-value 0.2407). The positive rate for age groups 30–39 and 40–49 were not largely changed at 3.4% (5/149) and 4.7% (12/257).

Large-scale seroepidemiologic surveillance of the anti-S positive rate

The anti-S antibodies of the August and December cohorts were analyzed by the anti-S ELISA. The presence of anti-S antibody indicates the vaccination and/or infection history. With the cut-off of 0.3 for the 40-fold serum dilution, the positivity rates were 38.7% (387/1,000 sera) and 90.8% (908/1,000 sera) in the August and the December cohorts. In the August cohort, the anti-S positive rates for the age groups 60–69 and 70–83 were relatively high at 70.7% (123/174) and 90.2% (37/41), respectively. The December cohort demonstrated a flattened positive rate at a high level (87.2%–100%) across all of the age groups tested. As expected, majority of the anti-N-positive sera were also anti-S-positive, with one exception for the August cohort and another for the December cohort.

Quantitative analysis of the anti-S antibody in the two cohorts

We quantitatively analyzed the anti-S positive sera in the August 2021 and December 2021 cohorts to estimate by calculating the area under the curves (AUCs) for the plot of the optical density against the dilution rate, and then compare the cohorts' anti-S antibody levels. The August 2021 cohort's distribution showed a significantly higher level of AUCs with the median 13.8 compared to that of the December 2021 cohort with the median 8.8 (Mann-Whitney U-test).

We further analyzed the distributions of the anti-S antibody titer by separating the age groups. There was no significant difference in the distribution among age groups in the August cohort by the Kruskal-Wallis test, whereas the distribution in the December cohort showed significant differences among the age groups, showing a clear tendency of decreased anti-S antibody levels in the older age group (70–79), indicating that the lower anti-S antibody titer did affect infection.

Neutralization activity of the anti-S-positive sera from the August 2021 cohort

To analyze the relationship between the anti-S antibody and neutralizing activity against SARS-CoV-2, the neutralization antibody titers of the anti-S-positive subset (n=387) from the August 2021 cohort were quantitatively evaluated. Although the distributions appeared similar with the same median value, i.e., 8, the Mann-Whitney U-test result indicated that the neutralizing titers for D614G were significantly higher than those for the Delta variant. The neutralization positivity rate in the anti-S-positive subset also showed a similar result in that there were slightly higher rates for D614G and Delta at 85.5% and 77.3%, indicating the efficacy of the two-dose vaccine for the Delta variant as well. The correlation plot between the anti-S AUC and the neutralizing antibody titer showed the positive correlation with the Spearman's rank correlation factor 0.78 and 0.77 for SARS-CoV-2 D614G and Delta, respectively.

Neutralizing activities of the sera collected in December 2021 against the Delta and Omicron variants

Detailed analysis of the December 2021 cohort is especially important because it represents the status after the Delta pandemic and before the Omicron pandemic. Thus, we analyzed the neutralization positivity rate against the Delta and Omicron variants in all 1,000 sera of the December 2021 cohort. The neutralization positivity rate against the Delta variant was 78.7% (787/1,000) in the December 2021 cohort, implying effective immunity was conferred to residents. In contrast, the neutralization positivity rate against the Omicron variant was quite low at 36.6% (366/1,000), implying the vulnerability of the cohort against infection by the Omicron variant.

A further analysis of the same data with a separation of age groups revealed that the neutralization positivity rate against the Delta variant tended to be lower in older age groups. The same trend was also observed in the neutralization positivity rate against the Omicron variant, and in the oldest age group (70–79 yrs) only 15.0% of the sera demonstrated the neutralization activity.

The comparison of the anti-S antibody titers between the neutralization-positive and -negative sera revealed that the neutralization-positive group had clearly higher titers of anti-S antibody in both the Delta and Omicron cases, supporting our speculation that the neutralization activity of the sera was attributed to the anti-S antibodies.

Lastly, we focused on the anti-N-positive (n=39) and anti-S positive (n=908) subsets of the December 2021 cohort. The neutralization-positive rate against the Delta and the Omicron variants for the anti-N positive subset were 89.7% and 59.0%, respectively. These values were slightly higher than those for the entire set of 1,000 sera or those for the anti-N negative subset (78.3% and 35.7% for the Delta and the Omicron variants), indicating superior immune responses in the individuals with a history of SARS-CoV-2 infection. The neutralization positive rate for the anti-S positive subset were 86.2% and 40.3% against the Delta and the Omicron variants, respectively.

4. Conclusion

In the present study, we analyzed totally 2,000 sera in August (n=1,000) and December (n=1,000) 2021 collected from individuals who underwent a health check-up to elucidate the current status in Hyogo prefecture of Japan. The anti-N seropositive rate were 2.1% and 3.9% in August and December 2021, demonstrating a Delta variant endemic during that time; it was approximately twofold higher than the rate based on the PCR-based diagnosis. The anti-S seropositive rate was 38.7% in August and it reached 90.8% in December, in concordance with the vaccination rate in Japan. In the December cohort, 78.7% of the sera showed neutralizing activity against the Delta variant, whereas that against the Omicron was much lower at 36.6%. These analyses revealed that effective immunity against the Delta variant was established in December 2021, however, prompt three-dose vaccination is needed to overcome Omicron's outbreak.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第 3194 号	氏 名	任 振霄
論文題目 Title of Dissertation	日本でのCOVID-19大規模血清疫学調査：デルタ株に対する中和抗体は得られているがオミクロン株に対しては得られておらず、オミクロン株の流行抑制には3回目のワクチン接種が必要である。 Large-scale serosurveillance of COVID-19 in Japan: Acquisition of neutralizing antibodies for Delta but not for Omicron and requirement of booster vaccination to overcome the Omicron's outbreak.		
審査委員 Examiner	主 査 Chief Examiner 勝二 郁夫 副 査 Vice-examiner 古屋敷智之 副 査 Vice-examiner 上田佳秀		

（要旨は１，０００字～２，０００字程度）

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The candidate, having completed studies on COVID-19, with a specialty in the serosurveillance of COVID-19 and the neutralizing antibodies, and having advanced the field of knowledge in the area of Clinical Virology, is hereby recognized as having qualified for the degree of Ph.D. (Medical).