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[Original article]

Orthogonal antibody testing for COVID-19 among healthcare workers in a non-epidemic place and time : Japan's Iwate Prefecture, May 18-31, 2020

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Abstract

Of the 47 prefectures in Japan, Iwate had the fewest cases of coronavirus disease 2019 (COVID-19), with the first diagnosis officially confirmed on July 28, 2020. A baseline serological survey of COVID-19 antibodies is essential to accurately evaluate an epidemic outbreak. The primary purpose of this study was to determine pre-epidemic prevalence of COVID-19 antibodies among healthcare workers, using two laboratory-based quantitative tests. In addition, a point-of-care (POC) qualitative test, rapid, simple, and convenient for primary care clinics, was compared with the laboratory-based tests. All antibody tests were performed on serum from 1,000 healthcare workers (mean age, 40 ± 11 years) in Iwate Prefectural Central Hospital, May 29-31, 2020. A COVID-19 case was defined as showing positive results in both laboratory-based quantitative tests. None of 1,000 samples had positive results in both of the laboratory immunoassays. The POC test showed positive results in 33 of 1,000 samples (3.3%) (95% confidence interval : 2.19-4.41), but no samples were simultaneously positive in both laboratory-based tests. In conclusion, COVID-19 cases were not serologically confirmed by a baseline control study of healthcare workers at our hospital in late May, 2020. Moreover, the POC qualitative test may offer no advantage in areas with very low prevalence of COVID-19, due to higher false-positive reactions compared with laboratory-based quantitative immunoassays.

Key words : antibody test, COVID-19, Iwate, SARS-CoV-2, seroprevalence

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread rapidly worldwide, affecting human health and social life[1-3)]. In Japan, the first COVID-19 case was confirmed in January 2020, and the total number of cases had reached 83,010 as of September 30, 2020[4)]. Among the 47 prefectures of Japan, only Iwate, a northeastern prefecture with a population of 1,227,647 (as of April 2020), had no reported COVID-19 cases until the first confirmed diagnosis on July 28, 2020, rising only to 23 cases as of September 30, 2020[5)].

To prevent in-hospital infection spread, a serological survey for COVID-19 among frontline health-

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care workers, at high risk of exposure, may be beneficial[6]. However, the true prevalence of COVID-19 remains unclear in Iwate because limited availability of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) diagnostic tests made it likely that some asymptomatic cases would be missed. Pre-epidemic prevalence of COVID-19 antibodies would allow comparisons with subsequent data to estimate infection trends and better address the health and social issues brought about by COVID-19. An orthogonal testing strategy with two or more laboratory-based quantitative immunoassays with very high specificity (99.5% or greater) has been useful in populations with a very low prevalence of COVID-19[7]. Simpler point-of-care (POC) antibody tests are useful in areas with a high-prevalence of COVID-19[8]. However, inadequate specificity may be problematic in low-prevalence areas, and their efficacy has not been studied in areas with no reported cases.

The primary purpose of this study was to investigate the prevalence of COVID-19 antibodies among healthcare workers at a tertiary hospital in northeastern Japan - where no official cases had been confirmed prior to July 28, 2020 - using two laboratory-based, high-specificity tests in an orthogonal analysis. Additionally, we also compared the accuracy of POC antibody tests with that of the quantitative COVID-19 antibody tests.

Materials and methods

Study design

Iwate Prefecture, with 1.2 million residents, is on the Pacific coast of northeastern Japan. To determine the seroprevalence of COVID-19 in our region, we retrospectively evaluated COVID-19 antibodies in serum from healthcare workers at Iwate Prefectural Central Hospital in the city of Morioka (Figure 1). The hospital, which has 685 beds, with a daily average of 1,100 outpatients and 534 inpatients in 2019, is one of the core medical institutions in Iwate Prefecture.

The study protocol was approved by the ethics committee of Iwate Prefectural Central Hospital, Iwate, Japan (approval number 343), in accord with the World Medical Association Declaration of Helsinki.

Study population and antibody tests

Blood samples were obtained from the annual

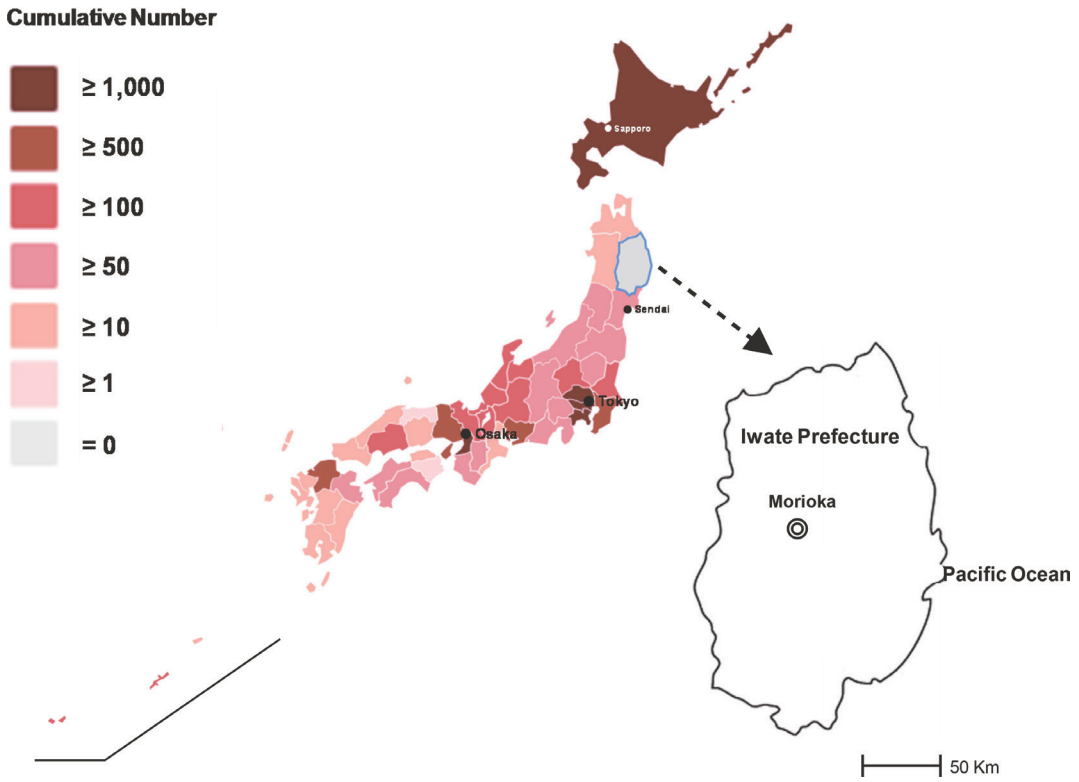

Fig. 1. The location of Iwate Prefecture and Iwate Prefectural Central Hospital. Number of COVID-19 cases by prefecture as of May 31, 2020, based on statistics from the Japanese Ministry of Health, Labour and Welfare (Ref. 4).

health checkups of 1,302 healthcare workers (physicians, nurses, pharmacists, radiographers, laboratory technicians, and medical office workers) on May 18-29, 2020, and stored at −20°C. Serum samples (n = 1,000) from employees from whom informed consent was obtained were analyzed for antibodies to SARS-CoV-2 using laboratory-based quantitative and POC qualitative tests on May 29-31, 2020.

Two laboratory-based quantitative tests that were approved by the US Food and Drug Administration were used : Abbott Architect® SARS-CoV-2 IgG Assay (chemiluminescent microparticle immunoassay ; sensitivity, 100% ; specificity, 99.6%) (Abbott Laboratories, Abbott Park, IL, USA)[7] and Roche Elecsys® Anti-SARS-CoV-2 RUO Assay (electrochemiluminescent immunoassay ; sensitivity, 100% ; specificity, 99.8%) (Roche Diagnostics, Basel, Switzerland)[7]. POC qualitative testing was performed with the Instant-view® IgG/IgM Antibody COVID-19 test (lateral flow chromatographic immunoassay ; sensitivity, 97.8% ; specificity, 94.6%) (Alfa Scientific Designs, Poway, CA, USA). All tests were conducted at room temperature and according to each manufacturer's instructions. Results were read visually after 10 minutes. Weak signals for IgM and IgG indicated a positive result.

A diagnosis of COVID-19 infection was made only if positive in both laboratory-based immunoassays ; a sample receiving only one positive result was considered noninfected. The prevalence of COVID-19 was determined by dividing the number of infected cases by that of samples. Continuous variables were expressed as mean ± SD, and categorical variables were expressed as numbers and percentages. The 95% confidence intervals (95% CIs) for the positive results in the tests were presented by the Wald method using Microsoft Excel.

Results

A total of 1,000 serum samples (from 264 men and 736 women ; mean age, 40 ± 11 years) were analyzed in this study. The laboratory-based immunoassays detected positive results in 4 of 1,000 samples (0.4%) (95% CI, 0.01-0.79) : Abbott's assay in 4 samples (0.4%) (95% CI, 0.01-0.79) and Roche's assay in 0 (0%) (Table 1). According to the study design that required positive results in both tests, no cases were confirmed positive (Table 2).

In contrast to the quantitative immunoassays, the POC qualitative test showed positive results with 33 of 1,000 samples (3.3%) (95% CI, 2.19-4.41). No sample had a positive result in both laboratory

Table 1. Results of the POC qualitative and laboratory-based quantitative tests for the detection of COVID-19 antibodies.

	POC qualitative test		Laboratory-based quantitative test	
	Alfa POC test		Abbott's Assay	Roche's Assay
Sample number	IgM	IgG	IgG	IgM + IgG
22	N	P	N	N
28	P	N	N	N
32	P	N	N	N
149	P	N	N	N
169	P	N	N	N
227	P	P	N	N
262	P	N	N	N
284	P	N	N	N
286	P	N	N	N
306	N	P	N	N
374	N	P	N	N
382	P	N	N	N
393	P	N	N	N
399	P	N	N	N
409	P	N	N	N
445	P	N	N	N
498	N	P	N	N
545	P	P	N	N
552	N	P	N	N
558	P	N	N	N
571	N	P	P	N
576	N	N	P	N
634	P	N	N	N
694	N	P	N	N
698	P	N	N	N
700	P	N	N	N
726	N	N	P	N
830	N	N	P	N
857	P	N	N	N
882	N	P	N	N
885	P	N	N	N
887	P	N	N	N
904	P	N	N	N
908	P	N	N	N
914	P	N	N	N
926	P	P	N	N
Total number	33 (IgM or IgG)		4	0
Positive rate (95% CI)	3.3% (2.19-4.41)		0.4% (0.01-0.79)	0%

CI, confidence interval ; COVID-19, coronavirus disease 2019 ; N, negative ; P, positive ; POC, point-of-care.

Table 2. Orthogonal comparison of Abbott's and Roche's assays.

	Roche's assay		
	Positive	Negative	Total
Abbott's assay			
Positive	0 (0%)	4 (0.4%)	4 (0.0%)
Negative	0 (0%)	996 (99.6%)	996 (99.6%)
Total	0 (0%)	1,000 (100%)	1,000

and POC tests (Table 1).

Discussion

To the best of our knowledge, this is the first study to evaluate the positivity rate of antibodies to SARS-CoV-2 in a region that had no officially confirmed cases of COVID-19 infection, data that would be necessary to meaningfully assess infection status after the epidemic. We qualitatively and quantitatively tested for SARS-CoV-2 antibodies in serum using two immunoassays and a POC test and found the following results. First, there were no positive results detected by both laboratory-based (Abbott and Roche) quantitative immunoassays. Second, because there were no positive results in Roche's assay, the results from the POC test were considered false positives. These results argue against using low-specificity COVID-19 antibody testing in an area with no known infection.

The COVID-19 epidemic in Japan reached its first peak on April 11, 2020, with a daily number of 714 new infected cases[4]. As of May 29, when blood sampling in this study concluded, the total number of cases in Japan was 16,650, of whom 891 individuals had died[4]. After the first peak on April 11, the number of daily COVID-19 cases gradually decreased and returned to January's early-onset levels. The overall number of infected cases was relatively small compared with those in other developed countries.

With the decline in cases in Japan, the focus has shifted from widespread testing for active COVID-19 to testing for antibodies. The serological tests to detect COVID-19 antibodies are crucial for individuals and to monitor the spread of the virus. In New York State, USA, which had an explosive growth of infections and was deemed an epicenter in early May 2020, the prevalence of COVID-19 was as high as 10%-15%[8]. Areas where the spread was relatively suppressed, such as Los Angeles County, California, USA, had rates <5%[9]. However, high rates in the USA shifted from New York to other regions in the early summer of 2020.

In Japan, the positive rate of COVID-19-specific IgG antibodies was 3.3% in 1,000 outpatients who visited Kobe City Medical Center General Hospital[10]. Blood samples obtained from 202 patients at community clinics in Tokyo showed a 5.9% rate of infection[11]. In late April, the Japanese Ministry of Health, Labour and Welfare (MHLW) examined the performance of five commercially available antibody test kits with donated blood collected in the Tohoku region (6 northeastern prefectures, including Iwate). This analysis showed that COVID-19 antibodies were detected in 2 of 500 samples, with a positive rate of 0.4%[4]. These data and our results suggest a low prevalence of COVID-19 in the Tohoku region, including our prefecture, compared with rates in Tokyo and Kobe City. Our results were also compatible with those of another MHLW antibody survey in Iwate's neighboring prefecture of Miyagi, which showed only a 0.03% positive rate[4].

Among the 47 prefectures in Japan, only Iwate had no reported COVID-19 cases until Iwate's first case was confirmed on July 28, 2020[5] ; it is probably rare for state-sized regions in other countries to be similarly without cases. As of May 29, over 6,000 inquiries for RT-PCR tests had been made to a local hotline from concerned individuals ; however, Iwate conducted only 730 tests, the lowest level in Japan[5].

While Iwate had no hospitalized cases of COVID-19, the prefecture had a very difficult time obtaining RT-PCR tests in the early weeks of the epidemic. Since neighboring prefectures such as Miyagi and Aomori[4] already had COVID-19 cases, Iwate possibly had cases, but they were not diagnosed due to the unavailability of tests. Throughout Japan, tests were initially reserved for symptomatic individuals or those with known exposure. In Iwate, test requests had to be approved by healthcare personnel, at least through the end of April.

Our hospital is in Morioka, the capital city of Iwate, and provides high-level medical research and services for the region's population. We connect with other clinics and hospitals through a medical network and play a central role in disease treatment and prevention. When the state of emergency was declared by Japan's Prime Minister on April 7[12], Iwate's residents were urged to stay in their homes. Because residents were not available for random blood-sample testing, we determined the prevalence of COVID-19 in our region by testing our hospital's healthcare workers. As of August 7, 2020, Japan had entered a second wave, with a daily number of

1,618 new infections and cumulative total of 44,770 cases[4]. In Iwate, the first case was reported on July 29, 2020, and 23 cases were diagnosed by September 30, 2020[5]. We considered that this testing would be essential to assess nosocomial infection and provide data to prevent the spread of infection and occurrence of the second wave of COVID-19. Furthermore, we believe that our study will provide data to inform epidemic models and make public policy decisions.

Several commercial COVID-19 antibody immunoassays are now available. In a region with very low prevalence, an antibody test with a high specificity (perhaps ≥ 99.5%) should be used, which would yield a higher positive predictive value[13]. This study's quantitative tests had a high specificity (99.6% and 99.8% in Abbott's and Roche's assays, respectively), and positive results were only detected in four (0.4%) samples in the former and no samples in the latter. There were more false-positive COVID-19 antibody results in the POC qualitative test, whose specificity was relatively low (94.6%). Antibody tests with high accuracy and consistent performance are needed not only in Iwate Prefecture but also throughout Japan to determine the prevalence of COVID-19.

This study has several limitations. First, it was a survey from a single medical institution with a relatively small number of blood samples. The participants in this study were healthcare workers ; thus, younger or older individuals were not enrolled. Second, positive and negative control tests are significant for the full validation of COVID-19 antibodies and assessment of nonspecific binding. In this study, attempts to use a positive or negative control test presented difficulties because of no identified infected cases and a stay-at-home order that affected the population. Third, the timing of COVID-19 antibody tests may be significant for accurate COVID-19 antibody detection. Because recent reports show the disappearance of antibody or a decrease in the titer after COVID-19 infection[14,15], repeated measurements of antibody would be warranted after a potent epidemic. Fourth, the POC tests for COVID-19 antibody are unreliable, and the development of accurate tests is essential as an epidemiological tool.

Conclusions

The baseline seroprevalence of COVID-19 antibodies was 0% among our hospital's healthcare workers. If this group can be taken as a representative sample of the population at large, our results suggest that Iwate Prefecture was unaffected by COVID-19 for a longer period than other Japanese prefectures. Such areas, with a low prevalence of COVID-19 cases, need tests with high specificity.

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Conflicts of interest disclosure

The authors declare no conflicts of interest.

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