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SARS-CoV-2 shedding and seroconversion among passengers quarantined after disembarking a cruise ship: a case series

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Summary

Background A cruise ship is a closed-off environment that simulates the basic functioning of a city in terms of living conditions and interpersonal interactions. Thus, the *Diamond Princess* cruise ship, which was quarantined because of an onboard outbreak of COVID-19 in February, 2020, provides an opportunity to define the shedding pattern of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and patient antibody responses before and after the onset of symptoms.

Methods We recruited adult (≥18 years) passengers from Hong Kong who had been on board the *Diamond Princess* cruise ship docked in Yokohama, Japan in February, 2020. All participants had been found to be negative for SARS-CoV-2 by RT-PCR 4 days before disembarking and were transferred to further quarantine in a public estate in Hong Kong, where they were recruited. Participants were prospectively screened by quantitative RT-PCR (RT-qPCR) of nasopharyngeal and throat swabs, and serum IgG and IgM against internal nucleoprotein and the surface spike receptor-binding protein (RBD) of SARS-CoV-2 at baseline (upon entering quarantine) and on days 4, 8, and 12 of quarantine.

Findings On Feb 22, 2020, 215 adults were recruited, of whom nine (4%; 95% CI 2–8) were positive for SARS-CoV-2 by RT-qPCR or serology and were hospitalised. Of these nine patients, nasopharyngeal swab RT-qPCR was positive in eight patients (89%; 57–99) at baseline. All nine patients were positive for anti-RBD IgG by day 8. Eight (89%; 57–99) were simultaneously positive for nasopharyngeal swab RT-PCR and anti-RBD IgG. One patient who was positive for anti-RBD IgG and had a negative viral load had multifocal peripheral ground-glass changes on high-resolution CT that were typical of COVID-19. Five patients (56%; 27–81) with ground-glass changes on high-resolution CT were found to have higher anti-nucleoprotein-IgG OD values on day 8 and 12 and anti-RBD IgG OD value on day 12 than patients without ground-glass changes. Six (67%; 35–88) patients remained asymptomatic throughout the 14-day quarantine period.

Interpretation Patients with COVID-19 can develop asymptomatic lung infection with viral shedding and those with evidence of pneumonia on imaging tend to have an increased antibody response. Positive IgG or IgM confirmed infection of COVID-19 in both symptomatic and asymptomatic patients. A combination of RT-PCR and serology should be implemented for case finding and contact tracing to facilitate early diagnosis, prompt isolation, and treatment.

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Introduction

A modern cruise ship is like a travelling city, with a common food and water supply, shared sanitation and air-conditioning systems, and a large population confined together.[1] The individuals are often from a middle-to-upper social class, with different cultures, immunisation backgrounds, and health statuses. The proximity of passengers and crew members in a semi-enclosed environment, with interactions in the dining halls, recreational rooms, spas, and pools, creates a unique environment for the person-to-person transmission of microbes. Contamination of commonly shared and frequently touched surfaces, food, or water supply or sanitation systems can occur and can cause considerable morbidity and mortality. Cruise passengers tend to be older people, with the 60–69-year-old group accounting for the majority,[2] who are more likely to have underlying chronic medical comorbidities and thus be more

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Research in context

Evidence before this study

We searched PubMed on March 14, 2020, with no date restrictions, for articles in English, using the terms "Covid-19", "coronavirus", "antibody", "viral load", "cruise ship", "quarantine", "shedding", and "seroconversion". Our search did not identify any reports on the prospective follow-up of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral shedding and seroconversion in a cruise ship setting. We identified one case report on the clinical presentation of two individuals on the *Diamond Princess* cruise ship, and an article estimating the effectiveness of public health measures on controlling the COVID-19 epidemic potential on a cruise ship.

Added value of this study

To our knowledge, this is the first cruise ship study of the clinical evolution and seroconversion from coronavirus disease 2019 (COVID-19) from last possible exposure to the pathogen to the end of the suspected incubation period. The study showed that asymptomatic individuals might seroconvert while carrying a high viral load and continue to shed the virus. Patients who had viral pneumonitis detected by high-resolution CT tended to have a higher antibody response. High-resolution CT also helped to establish a clinical diagnosis and detect cases of asymptomatic lung infection.

Implications of all the available evidence

Asymptomatic COVID-19 infection with continuous viral shedding makes infection control difficult. Therefore, a combination of RT-PCR and serology should be implemented for case finding and contact tracing in a community outbreak of COVID-19 to facilitate early diagnosis, prompt isolation, and treatment. High-resolution CT could also help to detect cases of asymptomatic infection.

susceptible to infection and associated complications than the general population. Common reported outbreaks on cruise ships have included respiratory infections with influenza A H1N1 and H3N2, Legionnaires' disease, and acute gastroenteritis due to norovirus.[1,3–6]

Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December, 2019, the virus has caused a pandemic affecting more than 4·7 million people, with more than 300 000 deaths in 229 countries.[7–10] On Jan 20, 2020, an 80-year-old man from Hong Kong boarded the *Diamond Princess* cruise ship in Yokohama, Japan, and disembarked in Hong Kong on Jan 25, 2020 (figure 1). On Feb 1, 2020, he was hospitalised with fever and tested positive for SARS-CoV-2. The cruise continued to Vietnam and Taiwan, then returned to Yokohama, at which time ten passengers were diagnosed with coronavirus disease 2019 (COVID-19),[11] and a 14-day quarantine order was imposed for all passengers and crew on board. Between Feb 5, and Feb 17, 2020, the Japanese Health Ministry tested all passengers by throat swab RT-PCR; individuals who tested positive were isolated in local hospitals. Individuals who tested negative were kept on board confined to their cabins and were only allowed out of the cabin for 1 h per day to exercise. The ship remained docked in the Port of Yokohama. By Feb 17, countries were allowed to air-evacuate citizens back to their home nations. Therefore, the Hong Kong Government chartered two flights on Feb 20 and Feb 21 to transfer passengers who had tested negative to Hong Kong for a further 14 days of quarantine in a newly completed public estate. As of May 14, 2020, 712 (19%) of the 3711 passengers and crew had been infected with SARS-CoV-2, and 13 deaths had occurred among those with confirmed infection.[12] We investigated the sequential SARS-CoV-2 shedding and the specific antibody response in this cohort of passengers quarantined in Hong Kong.

Methods

Study design and participants

Hong Kong citizens were air-evacuated from the *Diamond Princess* cruise ship on Feb 20 and Feb 21, 2020, and entered quarantine in the Chun Yeung estate, a newly built and vacant public estate in Hong Kong, on Feb 22. Each unit had a bathroom and kitchen and housed one to two people. During the 14-day quarantine period, they were placed in complete isolation. Only health-care workers from the Hong Kong Department of Health would visit daily to do health checks and to deliver meals. Family visits and contact with the general public were not allowed and individuals were asked to handle their own laundry. With approval by the Department of Health, we visited and collected samples from the individuals together with the aforementioned health workers. All adult passengers aged 18 years and older were screened and given the choice to join the study. There were no exclusion criteria. All participants provided written informed consent and could withdraw from the study at any point. The Institutional Review Board of the University of Hong Kong and Hospital Authority approved this study.

Procedures

Participants were screened by quantitative RT-PCR (RT-qPCR) for SARS-CoV-2 in nasopharyngeal, throat, and rectal swabs at baseline (Feb 22, 2020), day 4, day 8, and day 12, and tested for serum IgG and IgM against both internal nucleoprotein and the surface spike receptor-binding protein (RBD) of SARS-CoV-2, at baseline, day 4, day 8, and day 12.

The following participant data were entered into a database: name, Hong Kong personal identification number, age, sex, medical history by completion of a questionnaire, presenting symptoms and signs, and laboratory, radiological, virological, and serological

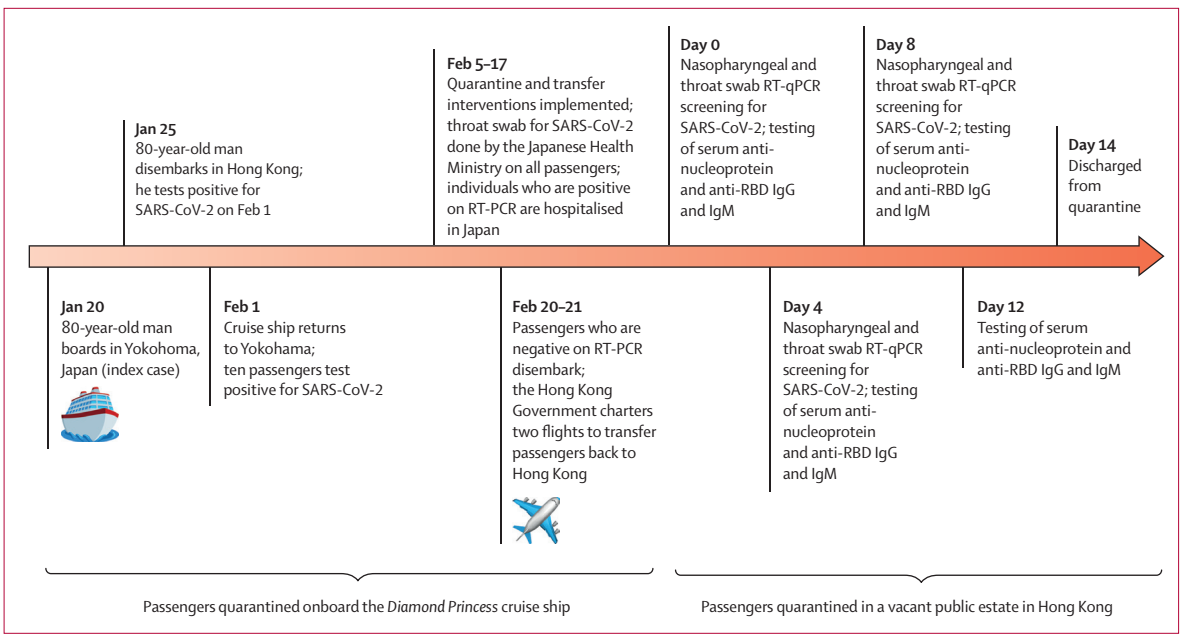

Figure 1: **Timeline of the outbreak of COVID-19 on the *Diamond Princess* cruise ship**
RBD=spike receptor binding domain. RT-qPCR=quantitative RT-PCR. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

findings. Participants who developed symptoms or who were found to have virological or serological evidence of SARS-CoV-2 infection at the time of screening were admitted to the Queen Mary Hospital or the Queen Elizabeth Hospital in Hong Kong for further investigation and treatment. All hospitalised participants underwent chest radiograph and high-resolution CT examination.

Nucleic acid extraction and RT-qPCR were done as previously reported.[13] Enzyme immunoassays for nucleoprotein and RBD and optical density (OD) cutoff values at 450 nm and 620 nm were established as previously described.[14] The cutoff value for positivity was based on the mean value plus 3 SDs of the negative control, using archived serum specimens collected in 2018 from 93 anonymous individuals without SARS-CoV-2 infection.[14]

Statistical analysis

Descriptive statistics are presented. Findings of the participants who tested positive for SARS-CoV-2 are presented as a case series. All data were analysed with SPSS (version 26.0).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

	Positive for SARS-CoV-2 (n=9)	Negative for SARS-CoV-2 (n=206)
Age, years, median (IQR)	58 (56–61)	64 (56–70)
Sex		
Female	6 (67%)	121 (59%)
Male	3 (33%)	85 (41%)
Underlying disease		
Diabetes	1 (11%)	23 (11%)
Hypertension	5 (56%)	58 (28%)
Chronic lung disease	1 (11%)	1 (<1%)
Ischaemic heart disease	1 (11%)	10 (5%)
Chronic renal disease	0	1 (<1%)
Thyroid disease	1 (11%)	5 (2%)
Neoplastic disease	1 (11%)	5 (2%)
Smoker	1 (11%)	18 (9%)

Data are n (%) unless otherwise indicated. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

Table 1: **Characteristics of 215 participants**

Results

Of 3711 passengers and crew members on board the *Diamond Princess* cruise ship, 369 passengers (10%) were from Hong Kong (appendix). The passengers returned to Yokohama on Feb 1, 2020, and were quarantined onboard for a further 20 days (figure 1). By Feb 20, 2020, 76 passengers from Hong Kong were hospitalised in Japan after testing positive for SARS-CoV-2 by throat swab RT-PCR, of whom two individuals died from complications of the infection

See Online for appendix

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Age, years	56	58	57	57	21	61	68	68	59
Sex	Male	Female	Male	Female	Female	Female	Female	Male	Female
Comorbidities									
Diabetes	No	No	No	No	No	Yes	No	No	No
Ischaemic heart disease	No	No	No	No	No	No	No	Yes	No
Hypertension	No	No	Yes	Yes	No	Yes	Yes	Yes	No
Hyperlipidaemia	No	No	No	No	No	Yes	No	Yes	No
Old pulmonary tuberculosis	No	No	No	Yes	No	No	No	No	No
Non-toxic multinodular goitre	No	Yes	No	No	No	No	No	No	No
Signs and symptoms									
Asymptomatic	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Chills and rigors	No	No	No	No	No	No	No	No	No
Cough	No	No	No	No	No	No	No	No	Yes (day 2)*
Sputum	No	No	No	No	No	No	No	No	No
Malaise	No	No	No	No	No	No	Yes (day –7)*	No	No
Myalgia	No	No	No	No	No	No	No	No	No
Diarrhoea	No	No	No	No	No	No	No	No	No
Rhinorrhoea	No	No	No	No	No	No	Yes (day –7)*	Yes (day –2)*	No
Fever	No	No	No	No	No	No	Yes (day –7)*	Yes (day –2)*	Yes (day 2)*
Initial laboratory findings									
Haemoglobin, g/dL (normal range 11·5–14·8)	12·2	12·5	13·1	11·7	9·5	13·2	12·5	14·2	14·0
White cell count, $\times 10^9$ per L (normal range 3·89–9·93)	5·24	7·20	7·19	6·49	6·42	6·40	7·20	7·90	3·36
Neutrophil count, $\times 10^9$ per L (normal range 2·01–7·42)	2·97	5·39	4·84	5·20	3·79	3·38	5·38	5·60	1·34
Lymphocyte count, $\times 10^9$ per L (normal range 1·06–3·61)	1·63	1·45	1·42	1·05	2·04	2·63	1·45	1·50	1·48
C-reactive protein, mg/dL (normal range <0·76)	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	<0·35	10·00	4·30
ALT, U/L (normal range 8–45)	17	22	50	15	24	23	30	44	36
LDH, U/L (normal range 143–280)	234	168	238	228	126	164	238	179	225
Creatinine, µmol/L (normal range 49–82)	92	52	104	59	57	45	95	82	50
Urea, mmol/L (normal range 2·9–8·0)	5·3	3·4	3·9	3·3	2·4	4·9	4·8	3·1	2·5
Creatine kinase, U/L (normal range 22–198)	183	52	177	134	60	45	90	Not measured	94
Initial radiological findings									
Chest radiograph	Normal	RLL haziness	Normal	Old granuloma	Normal	RML haziness	LLL haziness	Normal	RLL haziness
High-resolution CT	RLL GG	RUL and RLL GG	Normal	Old granuloma	Normal	RML GG	LUL and LLL GG	Normal	RLL GG
Virological findings (RT-PCR viral load, $\log_{10}$ copies per mL)									
Nasopharyngeal swab									
Baseline	ND	5·00	3·86	3·71	4·01	5·68	4·31	8·43	7·62
Day 4	ND	3·90	3·92	2·71	2·87	ND	ND	7·14	5·97
Day 8	ND	2·84	ND	ND	4·69	ND	ND	5·04	4·52
Day 12	ND	ND	ND	ND	3·30	ND	ND	3·57	3·60
Throat swab									
Baseline	ND	4·23	ND	ND	ND	ND	ND	7·02	6·76
Day 4	ND	3·95	ND	ND	ND	ND	ND	3·36	5·54
Day 8	ND	ND	ND	ND	ND	ND	ND	ND	5·45
Day 12	ND	ND	ND	ND	ND	ND	ND	ND	3·60

(Table 2 continues on next page)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
(Continued from previous page)									
Serological findings†									
Anti-nucleoprotein IgG									
Baseline	+	+	+	–	+	–	–	–	–
Day 4	+	+	+	–	+	+	+	–	–
Day 8	+	+	+	–	+	+	+	–	+
Day 12	+	+	+	–	+	+	+	–	+
Anti-nucleoprotein IgM									
Baseline	+	+	–	–	–	–	–	+	–
Day 4	+	+	–	–	–	–	–	+	–
Day 8	+	+	–	–	–	–	–	+	–
Day 12	+	+	–	–	–	–	–	+	–
Anti-RBD IgG									
Baseline	+	+	+	+	+	+	+	+	–
Day 4	+	+	+	+	+	+	+	+	–
Day 8	+	+	+	+	+	+	+	+	+
Day 12	+	+	+	+	+	+	+	+	+
Anti-RBD IgM									
Baseline	+	+	–	–	–	–	–	–	–
Day 4	+	+	–	–	–	–	+	–	–
Day 8	+	+	–	–	–	–	+	–	–
Day 12	+	+	–	–	–	–	+	–	–
Anti-nucleoprotein IgM/IgG ratio									
Baseline	0·37	0·75	0·09	0·50	0·04	0·37	0·11	0·07	0·13
Day 4	0·18	0·39	0·07	0·17	0·09	0·11	0·04	0·08	0·13
Day 8	0·35	0·21	0·05	0·17	0·04	0·08	0·02	0·07	0·12
Day 12	0·36	0·30	0·03	0·17	0·06	0·03	0·02	0·09	0·10
Anti-RBD IgM/IgG ratio									
Baseline	0·18	0·46	0·90	0·70	0·15	0·23	0·10	0·10	0·18
Day 4	0·07	0·18	0·16	0·26	0·28	0·13	0·12	0·09	0·12
Day 8	0·11	0·08	0·11	0·26	0·18	0·12	0·07	0·19	0·24
Day 12	0·11	0·10	0·06	0·29	0·14	0·08	0·07	0·11	0·11

ALT=alanine aminotransferase. LDH=lactate dehydrogenase. BLL=bilateral lower lobe. RUL=right upper lobe. RLL=right lower lobe. RML=right middle lobe. LUL=left upper lobe. LLL=left lower lobe. GG=ground glass changes. ND=not detectable. RBD=spike receptor binding domain. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. *Day –7 is Feb 15, 2020; day –2 is Feb 20, 2020; day 2 is Feb 24, 2020. †Serology results were calculated on the basis of optical density cutoff values established in a pre-pandemic cohort; nucleoprotein IgG cutoff=0·522, nucleoprotein IgM cutoff=0·177, RBD IgG cutoff=0·109, and RBD IgM cutoff=0·022; + indicates at or above the cutoff and – indicates below the cutoff.

Table 2: **Detailed profiles of nine patients with positive SARS-CoV-2 RT-PCR or serology**

(appendix). 293 passengers from Hong Kong who tested negative were allowed to disembark, of whom 65 decided to stay in Japan and 228 passengers (225 adults and three children) returned to Hong Kong for further quarantine. The three children were excluded from the study because their clinical data were not available for assessment. Ten passengers declined to join the study and remained well until discharge. Thus, 215 adult participants were enrolled. Baseline characteristics of participants who tested positive (n=9) and negative (n=206) for SARS-CoV-2 were well matched (table 1).

Nine (4%; 95% CI 2–8) of 215 participants were found to have evidence of SARS-CoV-2 infection and were hospitalised. These nine individuals were not related to each other; they boarded the ship in Hong Kong on Jan 25, 2020, when the index passenger had already disembarked and therefore had no contact with him (figure 1). At the last onboard COVID-19 testing by throat swab on Feb 17, 2020, all nine individuals tested negative. Both parents and the grandfather of patient 7 had tested positive for SARS-CoV-2 at the initial governmental screen in Japan. They were subsequently discharged from hospital in Japan after testing negative, and they returned to Hong Kong in March, 2020.

We have ordered the nine patients who tested positive for SARS-CoV-2 in the probable order of infection, according to their viral load and serological profile (table 2, figure 2A). Six (67%; 95% CI 35–88) patients remained asymptomatic (table 2). Patient 7 and patient 8 had had transient fever on Feb 15 (day –7) and Feb 20 (day –2), while still on board the cruise ship, but had not reached convalescence by the time of enrolment. Both

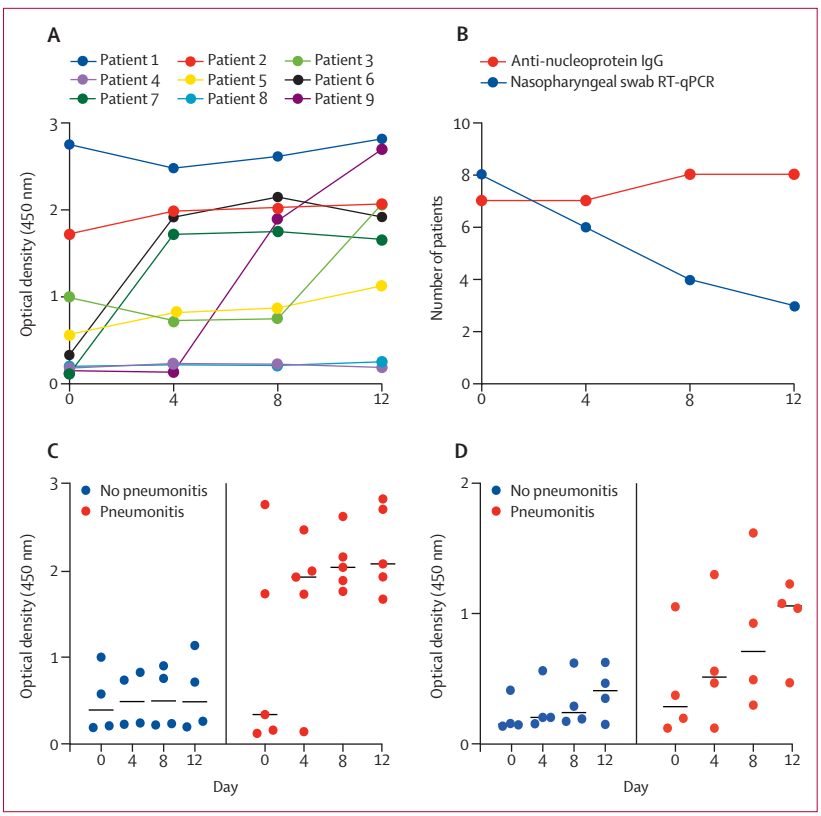

Figure 2: **Antibody responses in patients diagnosed with SARS-CoV-2 infection**
(A) Anti-nucleoprotein IgG response over time. (B) Number of patients with positive nasopharyngeal swab RT-qPCR and positive anti-nucleoprotein IgG over time. (C) Patients with positive anti-nucleoprotein IgG by pneumonitis status on high-resolution CT. (D) Patients with positive anti-RBD IgG by pneumonitis status on high-resolution CT. RBD=spike receptor binding domain. RT-qPCR=quantitative RT-PCR. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

patients were allowed to board the chartered flight to Hong Kong because they were afebrile when disembarking the ship. Of the three patients who had fever, one patient had concomitant symptoms of malaise and rhinorrhoea, one patient had an unproductive cough, and one had rhinorrhoea only (table 2). Comparison of the asymptomatic patients (n=6) with the symptomatic patients (n=3) showed that the asymptomatic patients were younger (median age 57 years [IQR 47–59] *vs* 68 years [59–68]), had a higher baseline nasopharyngeal viral load (7·62 $\log_{10}$ copies/mL [4·31–8·43] *vs* 4·66 $\log_{10}$ copies/mL [2·70–5·29]), and were more likely to be positive for anti-nucleoprotein IgG at baseline (four [67%] patients *vs* none).

Three (33%; 95% CI 12–65) of the nine patients with SARS-CoV-2 were men, and six (67%; 35–88) had underlying diseases, including hypertension, ischaemic heart disease, diabetes, hyperlipidaemia, old pulmonary tuberculosis, and non-toxic multinodular goitre. The median age was 58 years (IQR 57–65). The median (IQR) laboratory findings were: haemoglobin 12·5 g/dL (12·0–13·6), white cell count 6·49×10⁹ per L (5·82–7·20), neutrophil count 4·84×10⁹ per L (3·18–5·39), lymphocyte count 1·48×10⁹ per L (1·44–1·84), C-reactive protein 0·35 mg/dL (0·35–2·33), alanine aminotransferase 24 U/L (20–40), lactate dehydrogenase 225 U/L (166–236), creatinine 59 µmol/L (51–94), urea 3·4 mmol/L (2·8–4·9), and creatine kinase 92 U/L (54–166). Patient 5 had anaemia due to menorrhagia.

Patient 1 was the only patient with undetectable viral load on presentation (table 2), despite having a positive serological profile (figure 2A) and pulmonary changes on high-resolution CT (figure 3A) that suggested recent COVID-19. The other eight patients (89%; 95% CI 57–99) were positive on nasopharyngeal swab RT-PCR for SARS-CoV-2 at baseline. Patients 6 and 7 had transient detectable viral load at baseline, and patients 3 and 4 had transient detectable viral load on baseline and day 4. Patients 5, 8, and 9 had persistent detectable nasopharyngeal viral load through to day 12. Positive viral load could precede the onset of symptoms, as evidenced by patient 9. The median nasopharyngeal viral load at baseline was 4·31 $\log_{10}$ copies per mL (IQR 3·79–6·65), on day 4 was 2·87 $\log_{10}$ copies per mL (2·70–4·95), on day 8 was 2·84 $\log_{10}$ copies per mL (2·70–4·61), and on day 12 was 2·7 $\log_{10}$ copies per mL (2·70–3·44).

Three patients (33%; 95% CI 12–65) were found to be positive for SARS-CoV-2 on throat swab RT-qPCR (table 2). The median throat swab viral load at baseline was 2·70 $\log_{10}$ copies per mL (IQR 2·70–5·50) and at day 4 was 2·70 $\log_{10}$ copies per mL (2·70–3·66). The viral loads in the throat swabs were lower than those in the nasopharyngeal swabs at the same timepoints. Rectal swab viral load was negative in all nine patients. The nasopharyngeal SARS-CoV-2 RT-qPCR median viral load was higher in symptomatic patients than in asymptomatic patients (7·62 $\log_{10}$ copies per mL *vs* 3·86 $\log_{10}$ copies per mL).

Chest radiographs showed changes consistent with interstitial pneumonia in four patients (44%; 95% CI 19–73), with right lower lobe changes in patient 2, right middle lobe changes in patient 6, left lower lobe changes in patient 7, and right lower lobe changes in patient 9 (table 2, figure 3). Chest radiographs were normal in four patients (44%; 95% CI 19–73) and patient 4 had old granuloma from previous pulmonary tuberculosis. High-resolution CT (table 2, figure 3) showed ground-glass changes typical of COVID-19 in five patients (56%; 95% CI 27–81): patients 1 and 9 had right lower lobe ground-glass changes, patient 2 had right upper and lower lobe changes, patient 6 had right middle lobe changes, and patient 7 had left upper and lower lobe changes. Patients 3, 5, and 8 had normal chest radiograph and CT results, but tested positive on nasopharyngeal swab RT-PCR (table 2). Patients 3 and 5 were asymptomatic, whereas patient 8 developed fever and rhinorrhoea on day –2. Three patients (patients 1, 2, and 6) had pulmonary CT changes but were asymptomatic.

All nine patients were found to be positive for anti-RBD IgG (100% seroconversion) on days 8 and 12 (table 2).

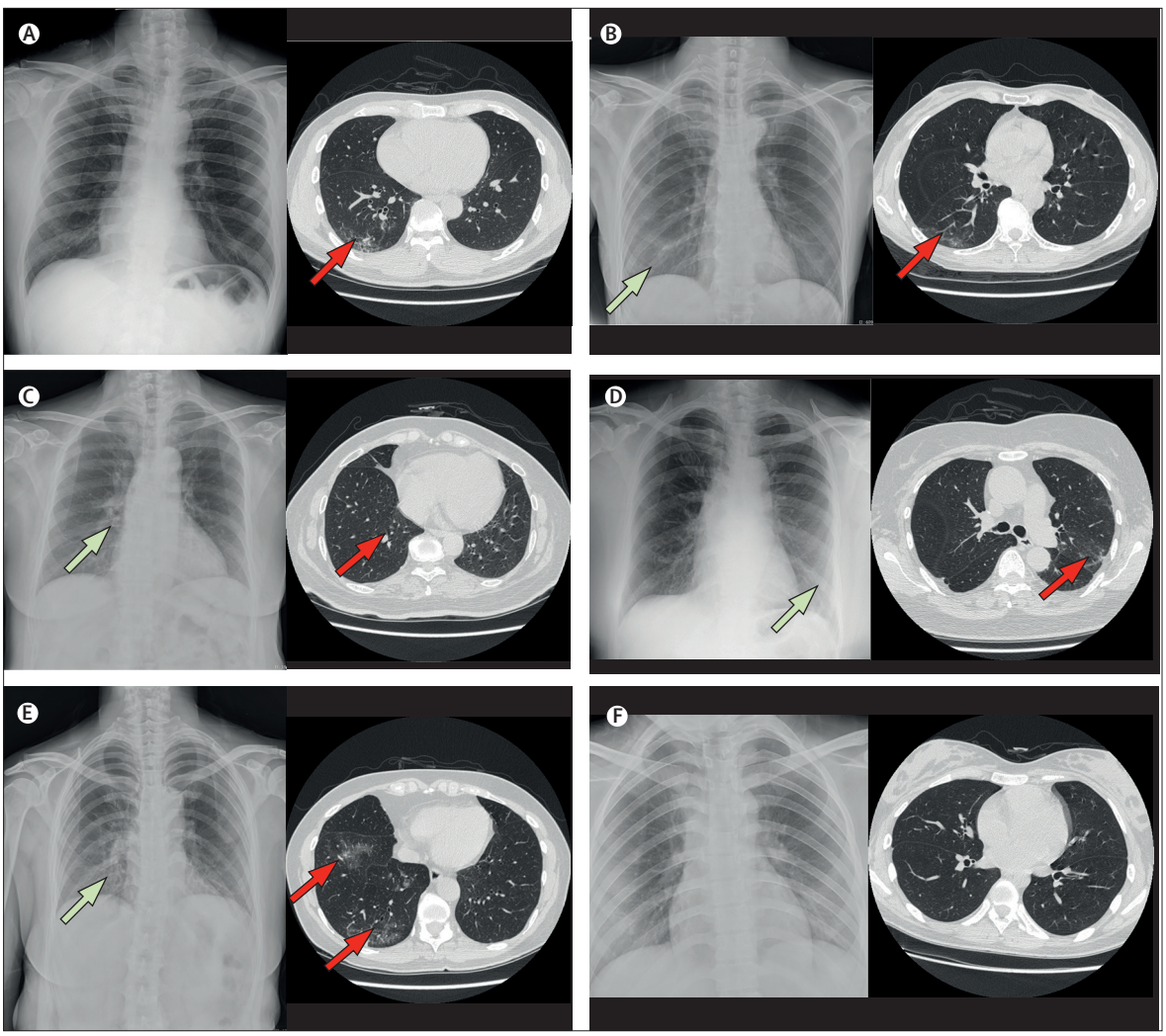

Figure 3: **Chest radiographs and high-resolution CT images of patients**
(A) Patient 1. (B) Patient 2. (C) Patient 6. (D) Patient 7. (E) Patient 9. (F) Patient 5 (normal chest radiograph and high-resolution CT). Green arrows indicate haziness on chest radiographs. Red arrows indicate ground-glass changes on high-resolution CT images.

Eight patients (89%; 95% CI 57–99) were simultaneously positive for anti-RBD IgG and nasopharyngeal swab RT-qPCR. Anti-nucleoprotein IgM and anti-RBD IgM were positive in three patients (33%; 95% CI 12–65). Patient 9 was the only patient who seroconverted for both anti-nucleoprotein and anti-RBD IgG on day 8, while remaining positive for nasopharyngeal and throat swab viral load. The IgM/IgG ratios for anti-nucleoprotein and anti-RBD are shown in table 2. All ratios were less than 1, suggesting that IgG was higher than IgM at all four timepoints and that the serological response for IgG was probably more robust than for IgM. No obvious pattern could be found to suggest the timing of infection.

The number of patients with positive anti-nucleoprotein IgG increased, with a corresponding decrease in positive nasopharyngeal swab RT-qPCR over the 12 days (figure 2). The five patients with high-resolution CT ground-glass changes suggestive of pneumonitis were found to have higher median anti-nucleoprotein IgG (OD 2·02 [IQR 1·81–2·38] *vs* 0·48 [0·21–0·85] on day 8; 2·06 [1·79–2·75] *vs* 0·47 [0·21–0·91] on day 12) and higher median anti-RBD (1·06 [0·76–1·16] *vs* 0·42 [0·26–0·55] on day 12) than the four patients without high-resolution CT changes.

Discussion

To our knowledge, this is the first study that investigates the real-time progression of a group of individuals who were initially negative for SARS-CoV-2 on RT-PCR after exposure to an outbreak of COVID-19 on a cruise ship. Nine individuals later tested positive on RT-PCR from nasopharyngeal swab, had high-resolution CT changes, or showed seroconversion, and were thus found to be infected with SARS-CoV-2. Despite the positive clinical

findings, six of the nine patients remained asymptomatic throughout the 14-day quarantine after leaving the ship (ratio of symptomatic to asymptomatic patients 1:2). If the cruise ship epidemic is a microcosm of the community outbreak scenario, then individuals with or without pneumonia could carry the virus for a long period but remain asymptomatic.[8,9] Asymptomatic patients with few or no comorbidities could spread the disease, whereas symptomatic patients represent only a small, but visible, proportion of total cases.[15] This finding is particularly important in densely populated cities like Hong Kong, with an average living space of 4·6 m² per person, equivalent to half a parking space.[16] Viral shedding from asymptomatic individuals could be transmitting the virus to others, making it difficult to identify and isolate index patients by contact-tracing for early quarantine. Transmission from asymptomatic individuals could explain in part the rapid increases in numbers of new cases in high-incidence countries such as the USA, Russia, the UK, Brazil, and European nations.[10]

SARS-CoV-2 could be spreading via respiratory droplets or through direct and indirect contact via the respiratory and gastrointestinal tracts.[17,18] Considering that most outbreaks on cruise ships are of respiratory and gastrointestinal infections, the risk of infection and associated morbidity and mortality in passengers increases with longer duration of travel and colder weather.[19,20] The period of exposure on the *Diamond Princess* cruise ship was 3 weeks, during which the passengers were unable to disembark, which is consistent with the high number of passengers (n=712) who contracted the infection.[12] The situation was likely to have been made worse by infected but asymptomatic crew members who continued to work unprotected, but who could have been shedding the virus. A recent modelling study[11] estimated that the initial basic reproduction number (R_0) of the outbreak on the *Diamond Princess* cruise ship was 14·8, before any infection control measures had been implemented and while the passengers were a completely naive group. The R_0 then decreased to 1·78 after the quarantine and transfer interventions were initiated (Feb 5–17, 2020).[11] This estimation of R_0 was lower than that of 2·2 (95% CI 1·4–3·9) that was suggested at the beginning of the outbreak in Wuhan,[21] although a higher R_0 of more than 3 has also been reported.[22] An R_0 of 2·2 for COVID-19 would be similar to that of SARS and influenza.[23] Nevertheless, evacuation of all passengers and crews early on in the outbreak would probably have prevented more infections, as suggested by the current findings. Because the screening test was done between early February and mid-February, before the air evacuation, we assumed that passengers could have acquired and been incubating the infection shortly before the test, and therefore tested negative, or they could have acquired the infection after the test. Based on this assumption, we screened 215 passengers by serial virological, serological, and radiological methods.

Our findings show a dynamic clinical presentation of SARS-CoV-2. Of the three diagnostic methods we used, the immunoassay for IgG and nasopharyngeal swab RT-qPCR were the most sensitive. Throat swab RT-qPCR was less sensitive;[24] it identified only the two patients who consistently had the highest viral load in nasopharyngeal swab RT-qPCR and did not identify the other six patients identified by nasopharyngeal swab RT-qPCR. The apparent low sensitivity of throat swab RT-qPCR might also account for the negative screening test done on board the ship by the Japanese Health Ministry. The three patients who were febrile felt transiently unwell, with concomitant symptoms of malaise, unproductive cough, and rhinorrhoea. The higher baseline nasopharyngeal swab viral load in symptomatic than asymptomatic patients in this study could be explained by the difference in time and degree of exposure to the virus, and might not be related to the symptoms.[25] Nevertheless, the persistent nasopharyngeal viral load in asymptomatic patients is a major concern for infection control. Rectal swabs were negative in all nine patients, which was expected because none of the patients presented with diarrhoea.[18] Important initial laboratory markers, including lymphocyte count, C-reactive protein, and lactate dehydrogenase, remained normal in these asymptomatic patients.

Seroconversion occurred in all nine patients and it was the only diagnostic marker that was positive for one patient in this cohort who had high-resolution CT changes characteristic of a recent SARS-CoV-2 infection. For those patients who might have acquired the infection earliest and only shortly before the screening test, they might have seroconverted with a high IgG titre and remained asymptomatic with a negative RT-qPCR, with high-resolution CT radiological evidence of SARS-CoV-2 pneumonitis with ground-glass changes. The immunological response could be explained by the lower respiratory tract infection consistent with pneumonia. Similar to influenza viral infection, patients with severe disease and pneumonia had the highest antibody response.[26] This finding could be related to the more severe viral infection resulting in a stronger induction of the host's adaptive immunity mediated by B and T cells.[27] Because SARS-CoV-2 and SARS-CoV have 90% amino acid homology for nucleoprotein and 73% amino acid homology for RBD, serum from patients with SARS-CoV might cross-react with our SARS-CoV-2 immunoassay, as shown by another group.[28] However, the cross-reactivity between SARS-CoV-2 and other human coronaviruses is expected to be lower, because the amino acid homology with nucleoprotein is less than 50%. Based on our previous study,[14] the sensitivity of the serology assay was 94% for anti-nucleoprotein IgG, 88% for anti-nucleoprotein IgM, 100% for anti-RBD IgG, and 94% for anti-RBD IgM. As stated in the Methods, the cutoff was based on a pre-pandemic general population cohort of

93 individuals without SARS-CoV-2 infection. Therefore, we expected that 1% would be outliers.[14]

The clinical presentation of the nine patients in this cohort ranged from mild to severe. Among the mild cases, patients could remain asymptomatic (patients 3, 4, and 5) or symptomatic (patient 8), with no lower respiratory tract involvement, as suggested by normal chest radiograph and high-resolution CT. Their anti-nucleoprotein and anti-RBD IgG levels were low. Patients with moderate infection had lower respiratory tract involvement, as suggested by the ground glass changes on high-resolution CT. Nevertheless, they could still remain asymptomatic (patients 1, 2, and 6) or become symptomatic (patient 7). These patients had higher anti-nucleoprotein and anti-RBD IgG than patients with mild disease. Patient 9, who acquired the infection more recently, developed symptoms upon hospitalisation. The infection was more severe, involving the lower respiratory tract, with ground glass changes on high-resolution CT. Seroconversion took place during the observation period, with anti-nucleoprotein and anti-RBD IgG still increasing at the end of the observation period.

Overall, anti-RBD IgG was the most sensitive serology marker, although the anti-nucleoprotein IgG response was stronger. The difference in anti-RBD IgG and anti-nucleoprotein IgG could be related to the severity of the viral infection, in which anti-RBD IgG was positive even in patients with upper respiratory tract infection, but at low OD values. Indeed, serology provides a feasible strategy to track SARS-CoV-2 infections.[29] We also investigated changes in IgM/IgG ratio for anti-nucleoprotein and anti-RBD and did not identify any trends that might reflect the timing of infection.

Similar to findings from one of the largest case series from China,[8] high-resolution CT provided a reasonable diagnostic tool, especially in asymptomatic patients. In this cohort, it was able to detect lung ground-glass opacities in five of nine patients, including the three patients with asymptomatic lung infection. Similar to other studies, right-side changes were more common.[30] Individuals with more severe radiological changes tended to have higher nasopharyngeal viral load. High-resolution CT could also detect an RT-qPCR-negative asymptomatic patient (patient 1) who had high IgG and IgM titres, suggestive of a recent infection. Nevertheless, individuals who develop only an upper respiratory tract infection could still have a normal high-resolution CT. Chest radiograph detected radiological changes in only four patients.

This study has several limitations. We did not have access to the data of patients who were hospitalised in Japan. We also did not have the cabin number and location of the participants when they were on board the ship, which could have provided some insight into how the infection was transmitted. Ten passengers did not give consent to participate in the study, and therefore we did not have their virological or serological data. Lastly, the study period was limited by the quarantine period and we could not follow up the participants beyond 14 days. The participants could have become RT-PCR-positive or antibody-positive after the quarantine period.

The cruise ship setting provided an unusual opportunity to study the viral shedding and seroconversion of SARS-CoV-2, mimicking the start of a community outbreak. Passengers who are exposed to the virus might seroconvert while remaining asymptomatic with a high viral load, and they could continue to shed the virus. High-resolution CT also helped to establish a clinical diagnosis and to detect cases of asymptomatic lung infection. A combination of RT-qPCR and serology should be done to screen for community outbreaks and effectively perform contact tracing.

Contributors

IF-NH, VC-CC, KK-WT, and K-YY were responsible for study design, data analysis, and writing up of the manuscript. IF-NH, VC-CC, XL, ART, DL-LH, KH-YC, DT-YH, S-CW, SS-ML, M-YC, MO-YT, TW-HC, T-CW, JW-MC, and C-SL were responsible for recruitment and clinical care of the patients. JF-WC and P-LH were responsible for data analysis and writing up of the manuscript. CC-YY, J-PC, SS-ML, JH-KC, RW-SP, AY-FF, RRZ, EY-WY, L-LC, CY-KC, K-HL, and K-HC were responsible for the laboratory analysis. SH-YL and TP-WL were responsible for interpretation of the radiological findings. All authors reviewed and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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