

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Sakurai A, Sasaki T, Kato S, et al. Natural history of asymptomatic SARS-CoV-2 infection. *N Engl J Med*. DOI: 10.1056/NEJMc2013020

Supplementary Materials and Methods

1. Description of the COVID-19 outbreak on the cruise ship Diamond Princess
2. The isolation and quarantine protocols at the hospital
3. Statistical analysis

Supplementary Table 1. Cp values of PCR tests of asymptomatic SARS-CoV-2 carriers over the course of their infections, who had at least one positive PCR in the hospital.

Supplementary Table 2. Demographics and co-existing medical conditions of asymptomatic SARS-CoV-2 carriers and presymptomatic patients, who were asymptomatic at the time of the first positive PCR test but subsequently developed symptoms.

Supplementary Figure 1. Flowchart of the cohort observed at the hospital.

Supplementary Figure 2. Crossing point (Cp) values of RT-PCR of asymptomatic SARS-CoV-2 carriers who had at least one positive PCR test result in the hospital.

Supplementary Figure 3. Crossing point (Cp) values of RT-PCR of asymptomatic SARS-CoV-2 carriers who had at least one positive PCR test result in the hospital, stratified by time to resolution of infection.

Supplementary Figure 4. Number of days from the first positive PCR test to the first of two serial negative PCR tests.

Supplementary Figure 5. Courses of 11 presymptomatic patients, who were asymptomatic at the time of the first positive PCR test but subsequently developed symptoms.

Supplementary Figure 6. Association between age and time to the resolution of infection.

1. Description of the COVID-19 outbreak on the cruise ship Diamond Princess

The British cruise ship Diamond Princess departed the Port of Yokohama in Japan on January 20, 2020. On February 1, a passenger from Hong Kong, who traveled for 5 days from Yokohama and left the ship at Hong Kong on January 25, tested positive for SARS-CoV-2. The ship was ordered to stay anchored offshore by the Japanese government for quarantine when it returned to Yokohama on February 3. Ten of 31 symptomatic passengers, crew and their cabinmates tested positive for SARS-CoV-2 on February 5, when the quarantine was implemented off the coast.¹ A total of 3,711 passengers and crew members were aboard at that time. In the early phase of the quarantine, only symptomatic passengers and their cabinmates were tested for SARS-CoV-2 by RT-PCR of nasopharyngeal or throat swabs. Beginning on February 11, indication for PCR testing was expanded to all passengers and crew members. At the time of testing, body temperature and clinical symptoms (cough, dyspnea, chest pain, sore throat, nasal discharge) were assessed by physicians and nurses from the quarantine stations, Japan Self-Defense Forces and disaster medical assistance team. Persons who tested positive for SARS-CoV-2 were classified into two groups; “symptomatic confirmed cases” and “asymptomatic confirmed cases” based on their symptomatic status at the time of testing.^{1,2} Those with a positive PCR test and their cabinmates were disembarked and transported to several hospitals for mandatory isolation and were observed until resolution of infection was achieved.

On March 5, after testing of 3,618 passengers and crew members and disembarkation of all of them, Ministry of Health and Labour and Welfare (MHLW) announced that there were 410 asymptomatic confirmed cases (i.e., asymptotically infected at the time of testing).³ Among them, up to 79 (19%) developed symptoms of COVID-19 during the follow-up and could be reclassified as presymptomatic. On April 30, MHLW announced that there were 311 asymptotically infected cases who remained asymptomatic throughout the long-term follow-up period.⁴

2. The isolation and quarantine protocols at the hospital

Fujita Health University Okazaki Medical Center, located 180 miles west from Yokohama, was a new hospital that was slated to open in April 2020. The hospital had not been approved as a medical facility and was not accepting any patients yet. Given the circumstances, a decision was made to accommodate some of the asymptomatic SARS-CoV-2 confirmed cases, both passengers and crew as well as their cabinmates who tested negative on the ship, at this hospital to continue their isolation and observation off the ship.

Asymptomatic confirmed cases were cohorted on two floors, while their cabinmates who tested positive on the ship were isolated in private rooms on a separate floor to prevent further transmission. They were monitored by physicians and nurses for their body temperature, oxygen saturation and symptoms twice a day. Those who developed signs and symptoms consistent with COVID-19 (i.e., fever $\geq 37.5^{\circ}\text{C}$, oxygen saturation $\leq 93\%$ without supplemental oxygen or new onset of respiratory symptoms) were transported to nearby acute care hospitals since the hospital had not been approved to provide medical care. PCR of nasopharyngeal swabs were performed approximately 48 hours after the previous positive test then repeated until two consecutive negative results collected ≥ 12 hours apart were obtained. This protocol was sanctioned at the time by MHLW as the criterion for releasing asymptomatic SARS-CoV-2 carriers (i.e., asymptomatic at the time of testing and remained so throughout) from isolation. All PCR tests were performed at a single government facility under the standard RT-PCR protocol endorsed by the National Institutes of Infectious Diseases. Crossing point (C_p) of 40 was used as the cutoff value for positivity. Meanwhile, cabinmates, all of whom had a negative PCR test on the ship, were tested 48 to 72 hours after their arrival to this hospital. If the test was positive, they were cohorted with asymptomatic SARS-CoV-2 confirmed cases. If the test was negative, they were isolated in private rooms for 14 days. The demographics and underlying medical conditions were extracted from the medical questionnaire

obtained upon their arrival to the hospital. This study was approved by the Institutional Review Board of Fujita Health University.

3. Statistical analysis

We analyzed binary variables with chi-squared test or Fisher's exact test and continuous variables with Mann-Whitney U test, respectively. All P values were two-sided. P values of <0.05 were considered statistically significant. Univariate logistic regression analysis was performed to assess the independent risk factors for being presymptomatic. Risk factors associated with delayed resolution of infection were assessed by multivariable regression analysis. Age, sex, passenger/crew, hypertension, diabetes mellitus, cardiovascular disease, chronic lung disease, chronic kidney disease, chronic liver disease and malignant tumor were included as explanatory variable in the regression model. Age was modeled with the use of restricted cubic splines to allow for non-linear associations with time to resolution of infection. The multiple regression model was internally validated using 150 resamples with bootstrap method. The degree of overfitting was quantified by optimism parameter in calibration plot, and there was no evidence of overfitting because optimism value was less than 0.2. The statistical analysis was performed using STATA 15.1 and R version 3.6.2.

Acknowledgments

We thank the staff of Okazaki Medical Center, Nagoya Quarantine Station, Disaster Medical Assistance Team (DMAT) and Dr. Yoshihiro Yamahata for their dedication in successfully bringing the mission to completion.

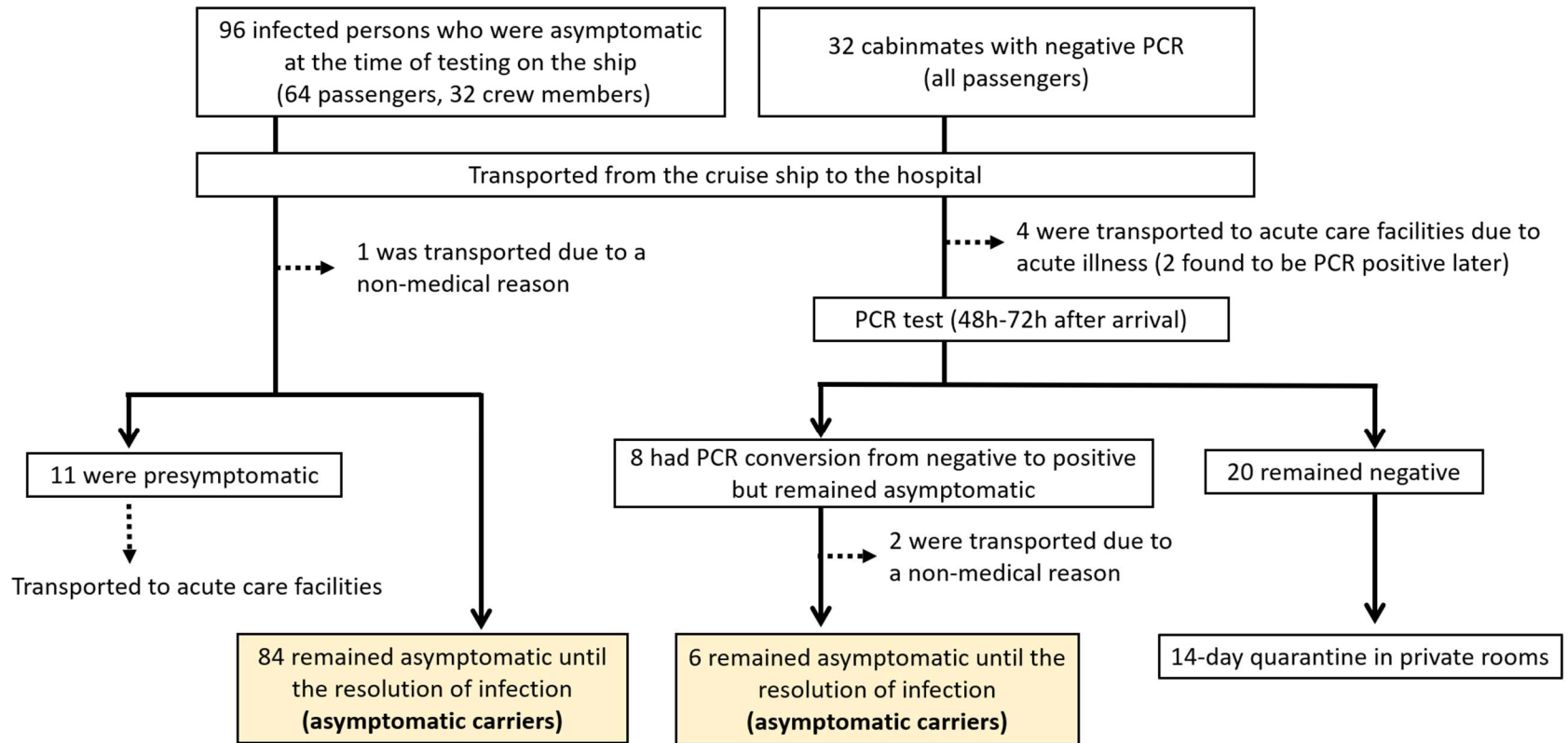
References

1. National Institute of Infectious Diseases, Field Briefing: Diamond Princess COVID-19 Cases, 19 February, 2020. <https://www.niid.go.jp/niid/en/2019-ncov-e/9407-covid-dp-fe-01.html>
2. National Institute of Infectious Diseases, Field Briefing: Diamond Princess COVID-19 Cases, 20 February, 2020. Update. <https://www.niid.go.jp/niid/en/2019-ncov-e/9417-covid-dp-fe-02.html>
3. Ministry of Health, Labour and Welfare, Official report on the cruise ship Diamond Princess, March 5, 2020. https://www.mhlw.go.jp/stf/newpage_09997.html
4. Ministry of Health, Labour and Welfare, Official report on the cruise ship Diamond Princess, May 1, 2020. https://www.mhlw.go.jp/stf/newpage_11146.html

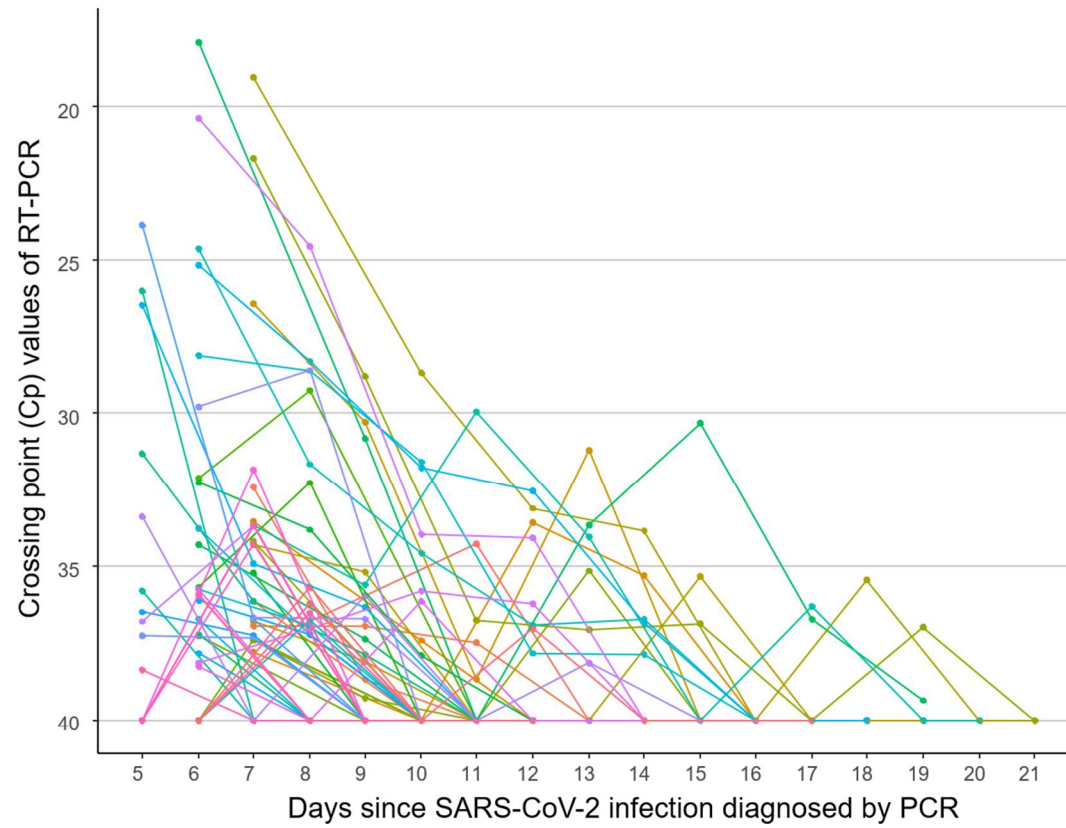
Supplementary Table 2. Demographics and co-existing medical conditions of asymptomatic SARS-CoV-2 carriers and presymptomatic patients, who were asymptomatic at the time of the first positive PCR test but subsequently developed symptoms.

	Asymptomatic carriers (N=90)	Presymptomatic patients (N=11)
Age (median)	59.5 (IQR 36-68, Range 9-77)	68.0 (IQR 60-74, Range 50-76)
Male	53 (58.9%)	7 (63.6%)
Passengers	58 (64.4%)	11(100%)
Co-existing medical conditions	24 (26.7%)	1*
Hypertension	18 (20.0%)	1*
Hyperlipidemia	3 (3.3%)	0*
Diabetes mellitus	8 (8.9%)	0*
Cardiovascular disease	2 (2.2%)	1*
Chronic lung disease	3 (3.3%)	0*
Chronic kidney disease	1 (1.1%)	0*
Malignant tumor	3 (3.3%)	1*
Chronic liver disease	0 (0%)	1*

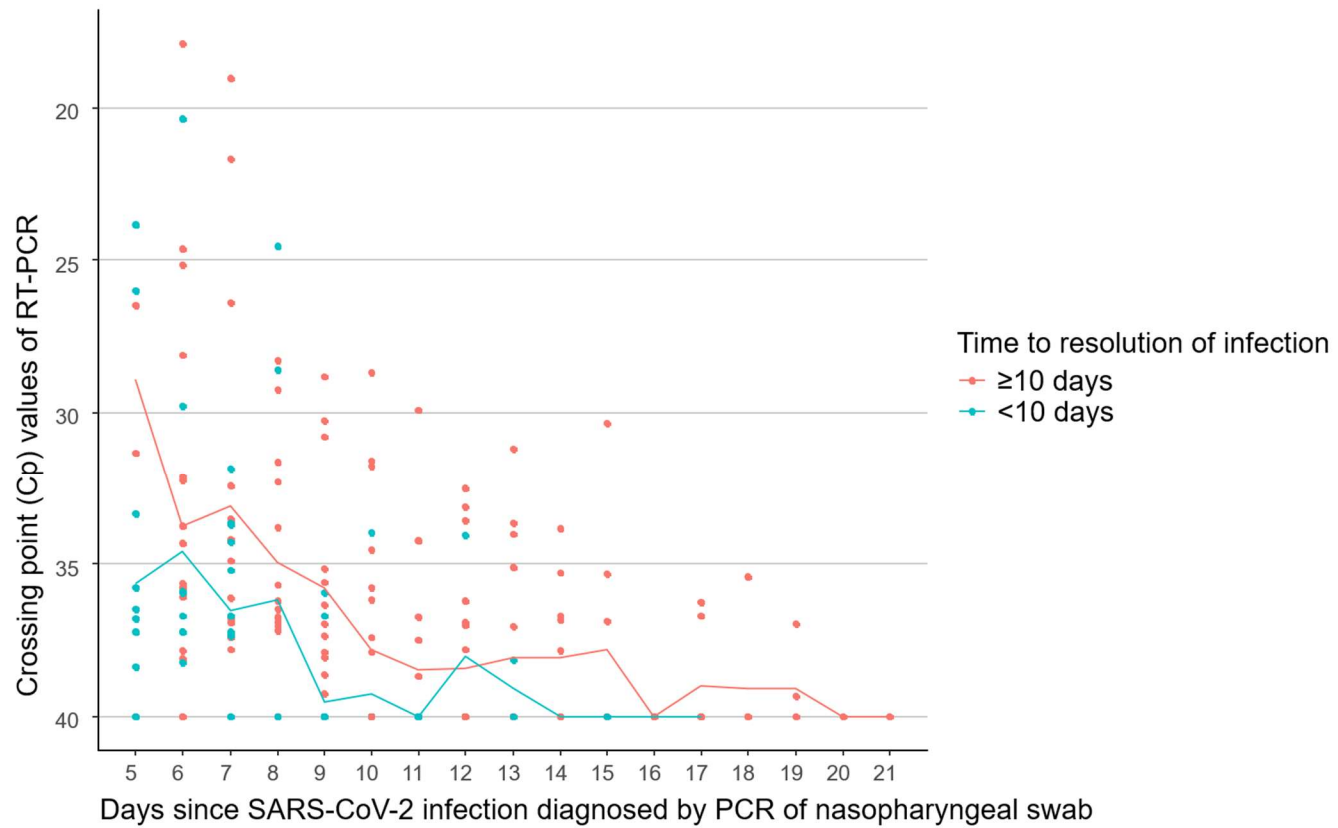
* Co-existing conditions were obtained from 4 of 11 in presymptomatic patients.



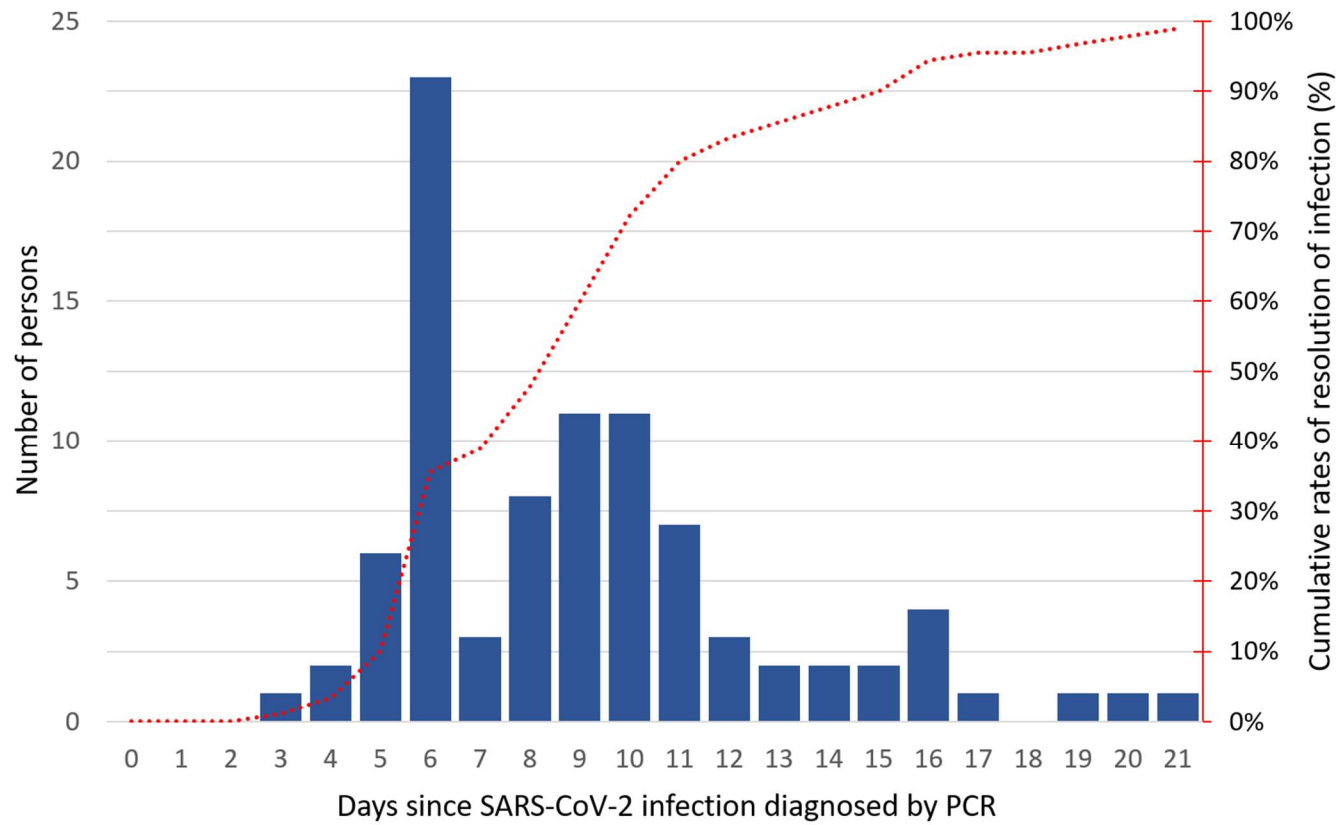
Supplementary Figure 1. Flowchart of the cohort observed at the hospital.



Supplementary Figure 2. Crossing point (Cp) values of RT-PCR of asymptomatic SARS-CoV-2 carriers who had at least one positive PCR test result in the hospital.



Supplementary Figure 3. Crossing point (Cp) values of RT-PCR for 90 asymptomatic SARS-CoV-2 carriers, stratified by time to resolution of infection.



Supplementary Figure 4. Number of days from the first positive PCR test to the first of two serial negative PCR tests.

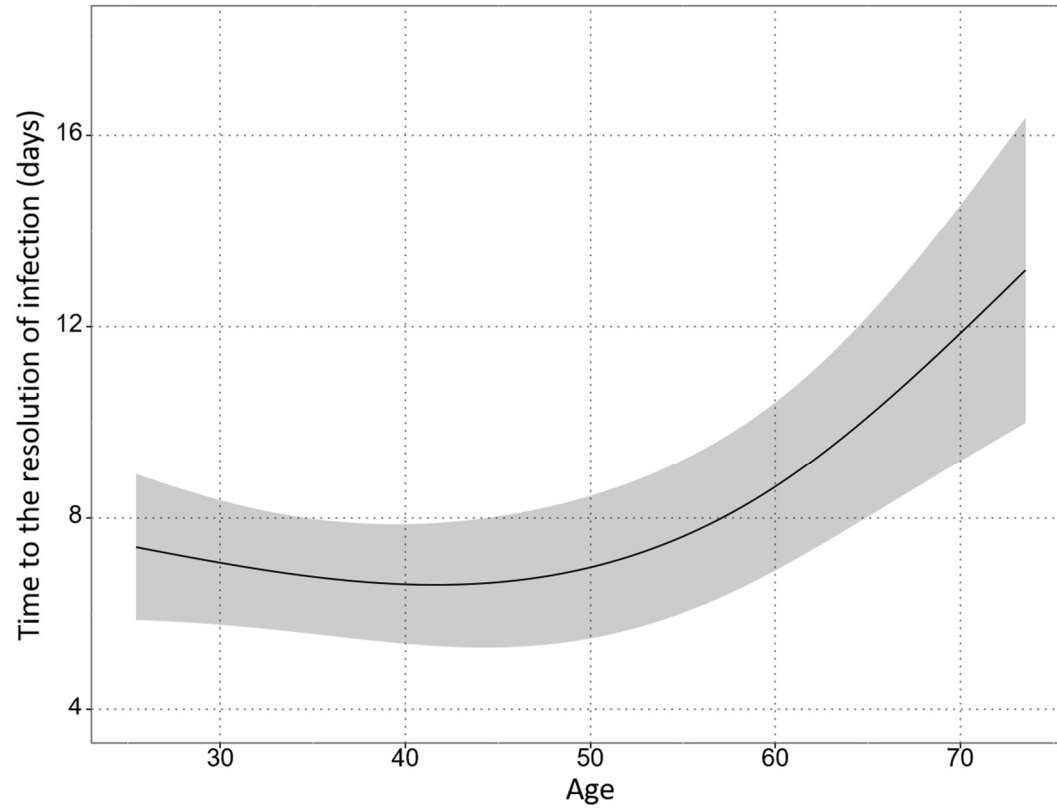
Patient	14-Feb		15-Feb		16-Feb		17-Feb		18-Feb		19-Feb		20-Feb		21-Feb		Reasons for transfer
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	
1	PCR+											■XX					fever, desaturation, cough
2		PCR+										■XX					desaturation
3		PCR+										■XX					fever, lethargy
4		PCR+										■XX					fever, desaturation
5		PCR+										■			XX		cough, sputum
6				PCR+								■	XX				fever
7					PCR+							■	XX				fever, sore throat
8					PCR+							■XX					desaturation, cough, sputum
9					PCR+									■XX			fever, chest pain
10					PCR+									■XX			desaturation, cough
11							PCR+							■XX			fever

PCR+, first positive PCR on the cruise ship

■, arrival from the cruise ship to the facility

XX, onset of clinical signs or symptoms consistent with COVID-19

Supplementary Figure 5. Courses of 11 presymptomatic patients, who were asymptomatic at the time of the first positive PCR test but subsequently developed symptoms.



Supplementary Figure 6. Association between age and time to the resolution of infection.