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BRIEF REPORT

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Coinfection with severe acute respiratory syndrome coronavirus-2 and other respiratory viruses at a tertiary hospital in Korea

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Abstract

Background: Studies have reported coinfection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the cause of coronavirus disease-2019 (COVID-19), with other viruses that cause respiratory tract infections (RTIs). We investigated the coinfection rate of SARS-CoV-2 and other RTI-causing viruses, and whether the cycle threshold (Ct) value of a real-time reverse transcriptase PCR (RT-PCR) differed when the coinfection occurred during the first wave of COVID-19 in Daegu, Republic of Korea, in 2020.

Methods: After performing PCR for SARS-CoV-2, we additionally tested for the presence of RTI-causing viruses to check for coinfection. Subsequently, we identified the specific coexisting respiratory viruses and calculated the coinfection rate. In addition, based on the coinfection status, we compared the Ct values obtained from RT-PCR for SARS-CoV-2 in patients who tested positive for COVID-19 PCR.

Results: Of 13,717 patients, 123 had positive results on COVID-19 PCR testing and six tested positive for an RTI-causing virus. Thus, the coinfection rate was 4.9%. There were no statistically significant differences in the mean Ct values of SARS-CoV-2 RT-PCR between coinfected and non-coinfected patients.

Conclusion: This study computed the coinfection rate of SARS-CoV-2 and RTI-causing viruses and revealed that the mean Ct values in SARS-CoV-2 real-time RT-PCR did not differ according to the coinfection status.

KEYWORDS
COVID-19, SARS-CoV-2, coinfection, real-time RT-PCR, cycle threshold

1 | INTRODUCTION

Since it was first reported in Wuhan, China, in December 2019, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the cause of coronavirus disease-19 (COVID-19), quickly spread across the globe; the disease burden is increasing with a global loss of GDP and health. The World Health Organization (WHO) declared the disease a pandemic in March 2020, and as of March 7, 2021, there were 116,166,652 confirmed cases and 2,582,528 deaths.[1] Although vaccines against SARS-CoV-2 have been developed and are being administered worldwide since December 2020,[2–4] the pandemic remains active. The cycle threshold (Ct) value of a real-time RT-PCR

SARS-CoV-2 test has been associated with disease severity and patient mortality.[5] Furthermore, some studies have explored the pathophysiology of SARS-CoV-2[6,7] as well as the multidisciplinary approaches for COVID-19 management as it affects most organ systems, including cardiovascular, neurological, and hematological systems.[8–10]

Besides SARS-CoV-2, other viral respiratory tract infections (RTIs) also have a detrimental impact on human mortality and morbidity and are one of the leading causes of death.[11] Although most cases of RTIs do not require additional treatment, Coronaviridae, including SARS-CoV-2, Paramyxoviridae, and Picornaviridae, may result in the development of secondary bacterial infections after RTI. Recently, a study promoting an understanding of RTI viruses, including SARS-CoV-2, was published for clinicians.[12]

Coinfection of SARS-CoV-2 and other RTI viruses is gaining attention. Several studies have reported the coinfection rates and changes in symptoms, the rate of hospitalization, and the length of hospital stay after coinfection.[13–22]

The first wave of COVID-19 infections occurred in Daegu, Republic of Korea, between February and May 2020. Here, we investigated specific RTI-causing viruses and their possible interactions with SARS-CoV-2. Further, we calculated the coinfection rate and variations in Ct obtained from real-time RT-PCR SARS-CoV-2 tests during the aforementioned period.

2 | MATERIALS AND METHODS

The flow diagram for subject allocation is shown in Figure 1. Here, we enrolled patients who underwent COVID-19 real-time RT-PCR (COVID-19 PCR) testing between February 28 and May 2, 2020, at the Yeungnam University Medical Center in Daegu. These patients either showed COVID-19 symptoms or were in close contact with COVID-19 patients.

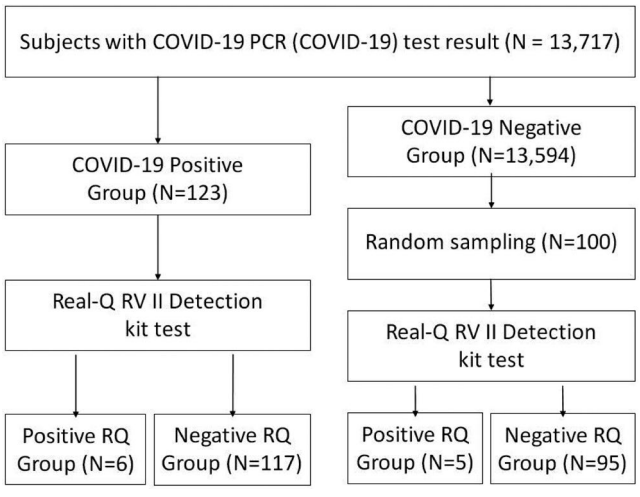

FIGURE 1 Flow diagram for subject allocation. Abbreviations: COVID-19, coronavirus disease 2019; PCR, polymerase chain reaction; RQ, Real-Q RV II Detection kit

This study was approved by the Institutional Review Board of Yeungnam University Medical Center (IRB No. 2020-03-115).

2.1 | Laboratory test for COVID-19 and RTI

The SARS-CoV-2 must be present for the diagnosis of COVID-19. At the Yeungnam University Medical Center, real-time RT-PCR tests were performed using nasopharyngeal swab samples to confirm COVID-19. The test kit used was Allplex 2019-nCoV Assay (Allplex; Seegene Co. Ltd, Seoul, Republic of Korea), which obtained an emergency use authorization (EUA) from the South Korea Ministry of Food and Drug Safety. The samples remaining after COVID-19 PCR tests were stored in a deep freezer at −70°C and subsequently analyzed using the Real-Q RV II Detection kit (RQ; Bioseum Co. Ltd, Seoul, Republic of Korea); the kit simultaneously detects 16 major RTI-causing respiratory viruses—adenovirus, parainfluenza virus 1/2/3/4, enterovirus, influenza virus A/B, coronavirus 229E/OC43/NL63, rhinovirus, respiratory syncytial virus A/B, metapneumovirus, and bocavirus. The RQ assay was performed in patients who tested positive and 100 randomly selected specimens from those who tested negative on the COVID-19 PCR test.

2.2 | Statistical analysis

The continuous variables were compared using Welch's two-sample t test; they were presented as median and interquartile range (IQR). The categorical variables were compared using Pearson's chi-square test or Fisher's exact test; they were presented as total count and percentage. Statistical significance was set at a p-value of 0.05, and p-values less than 0.05 were deemed statistically significant. All statistical analyses were performed using R statistical software version 4.0.3, and graphs were generated using the ggplot2 package in R.[23]

3 | RESULTS

3.1 | Demographic characteristics of the allocated subjects

During the study period, 13,717 individuals underwent COVID-19 PCR testing, and 123 tested positive. When the RQ assay was performed on the specimens of these 123 individuals, six (4.9%) tested positive and 117 (95.1%) tested negative (Figure 1).

To represent 13,594 individuals who tested negative on the COVID-19 PCR test, 100 specimens were randomly selected and analyzed using RQ assay. On analysis, five (5.0%) individuals tested positive and 95 (95.0%) tested negative (Table 1).

The percentage of RQ-positive patients did not significantly differ between COVID-19 PCR-positive and -negative individuals. Similarly, the male-to-female sex ratio also did not significantly differ between the two groups. However, the median age of the male and female individuals differed significantly.

TABLE 1 Demographic characteristics of COVID-19 PCR-positive and sampled COVID-19 PCR-negative subject groups

	COVID-19 PCR Positive	COVID-19 PCR Negative	*p*-value
N	123	100	
Female, median (%)	70 (56.9)	47 (47.0)	0.18
Male, median (%)	53 (43.1)	53 (53.0)	
Age, median (%)	60 (47.5, 67)	71.5 (59.75, 80)	<0.001[†]
Female, median (IQR)	59 (42, 65.75)	77 (64.5, 83)	<0.001
Male, median (IQR)	60 (53, 73)	66 (57, 75)	0.53
Real-Q RV II Detection kit			
Positive, median (%)	6 (4.9)	5 (5.0)	1
Negative, median (%)	117 (95.1)	95 (95.0)	

Abbreviation: IQR, interquartile range.

[†]Comparison using Pearson's chi-squared test, Welch two-sample *t* test, or Fisher's exact test, accordingly.

TABLE 2 Clinical and laboratory characteristics of the patients with Real-Q RV II Detection kit-positive specimens

Case	Sex	Age, years	Symptoms on arrival	COVID-19 PCR	Real-Q RV II Detection kit Virus group	Confirmed virus[#]
1	M	24	Sore throat, cough, and sputum	Positive	CoV 229E/OC43	Human coronavirus 229E
2	F	26	.	Positive	AdV (A-F)	Human mastadenovirus A
3	F	60	.	Positive	HRV (A-C)	Human rhinovirus B
4	F	50	Sore throat	Positive	FluA	Influenza A virus
5	F	56	Sore throat	Positive	HRV (A-C)	Human rhinovirus A
6	M	61	Sputum	Positive	AdV (A-F)	Human mastadenovirus A
1	M	54	General weakness	Negative	CoV 229E/OC43	Human coronavirus OC43
2	F	72	Fever, sputum, sore throat, and hemoptysis	Negative	AdV (A-F)	Human mastadenovirus A
3	M	40	Abdominal pain	Negative	CoV 229E/OC43	Human coronavirus 229E
4	M	85	Fever	Negative	HRV (A-C)	Human rhinovirus A
5	M	78	Dyspnea	Negative	HRV (A-C)	Human rhinovirus B

Abbreviations: AdV, adenovirus; CoV, coronavirus; FluA, influenza A virus; HRV, rhinovirus.

[#]Sequenced and then NCBI BLAST searched virus names of the Real-Q RV II Detection kit-positive specimen.

3.2 | Clinical characteristics of RQ-positive subjects

Table 2 shows the clinical characteristics of patients who tested positive on the RQ assay after testing positive (n = 123) or negative (n = 100) on the Allplex test. The sequence information shown in the last row was obtained by searching the website for the National Center for Biotechnology Information using the Basic Local Alignment Search Tool (BLAST).

3.3 | Laboratory analysis between Allplex and RQ

The Ct values for the target gene, used to determine positivity/negativity on the Allplex assay, are shown as box plots (Figure 2). The RQ test results for specimens that were positive on the COVID-19 PCR test are shown on the x-axis and their E, RdRP, and N gene Ct values, used for the Allplex assay, are shown on the y-axis. According to the box plots, the differences in the mean Ct values between RQ-positive (with coinfection) and RQ-negative (without coinfection) specimens were not statistically significant; P-values for the E, RdRP, and N genes were 0.91, 0.87, and 0.76, respectively.

4 | DISCUSSION

An array of colonized viruses exists in the supposedly sterile pulmonary environment[24,25]; however, the presence of these viruses may not cause an infection. Nevertheless, it is still necessary to detect these respiratory tract viruses to verify the assumption that they may cause a coinfection with SARS-CoV-2. Therefore, we performed RQ assays on patients who underwent SARS-CoV-2 testing for COVID-19.

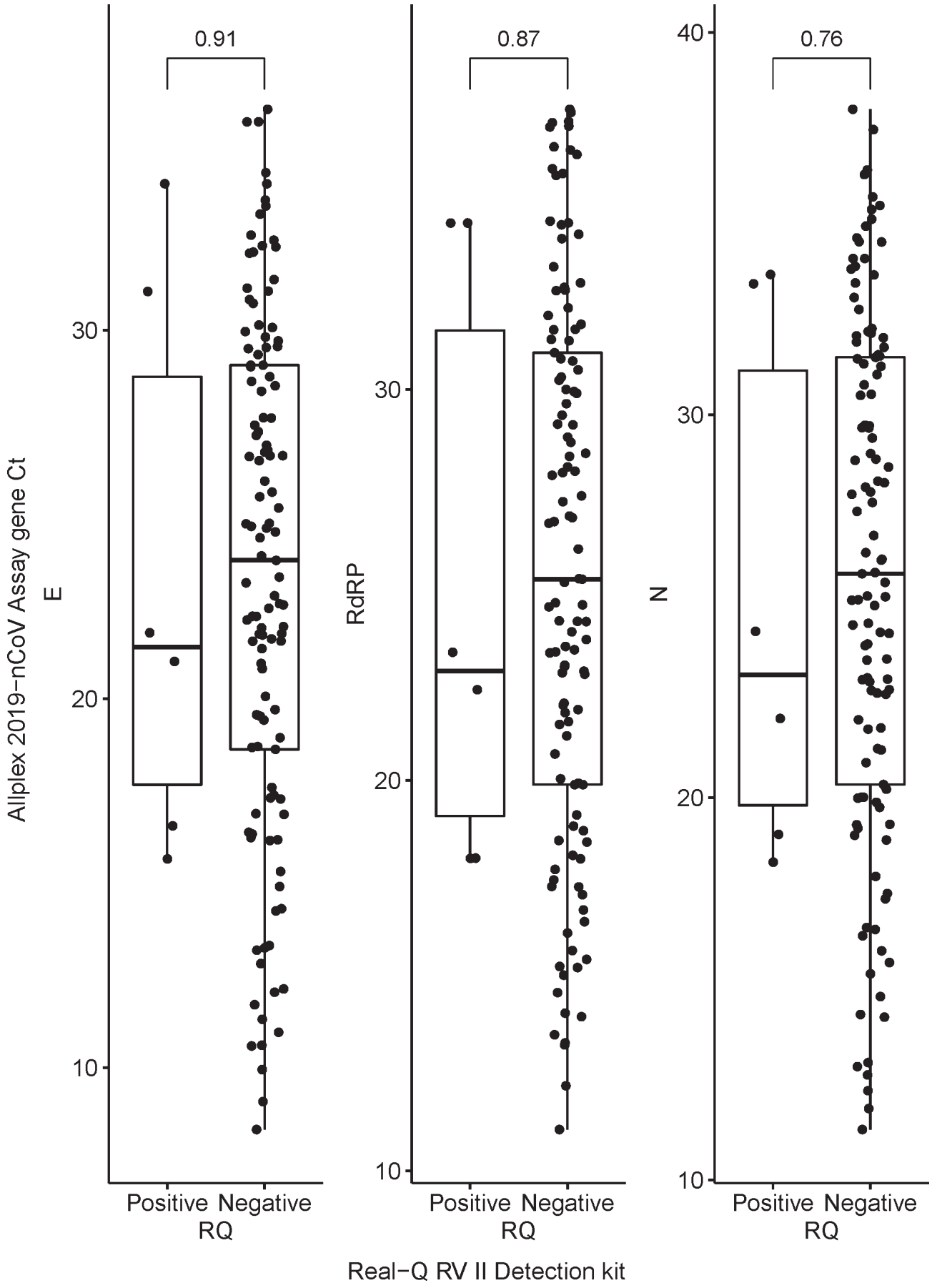

FIGURE 2 Box plots of E, RdRP, and N gene Ct values of Allplex 2019-nCoV assay according to positive or negative results of the Real-Q RV II Detection kit in the COVID-19 PCR-positive group. Notes: Comparison using the Wilcoxon test. Abbreviations: Ct, cycle threshold; E, envelope; N, nucleocapsid; RdRP, RNA-dependent RNA polymerase; PCR, polymerase chain reaction; RQ, Real-Q RV II Detection kit

Of the 123 patients who tested positive on the SARS-CoV-2 PCR test, 4.9% were coinfected with an RTI-causing virus. This was lower than the coinfection rates reported by Kim et al. (20.7%)[13] and Ling Ma et al. (15.6%).[26] While examining the coinfection rate, the fact that the time and regions differed across studies should be taken into account. Although we could not find studies confirming coinfection rates in different geographic locations in a single study period, different coinfection rates were found in East/Mid/West Asia, Europe, North Africa, and North/South America.[27] Furthermore, as mentioned in previous studies,[28,29] the results could have differed due to the differences in the test kits (such as Allplex and RQ test kits) and obtained specimens (such as nasopharyngeal swabs). Besides, there could be limitations related to the multiplex PCR test method, which simultaneously detects multiple respiratory viruses as opposed to a single virus.

According to a previous study, there was a disagreement between the detection of upper and lower respiratory viruses using nasopharyngeal swabs.[28] Karhu et al reported that in only 8 out of 24 patients, the virus which was detected in the trachea was found in the nasopharyngeal swab sample. However, in this study, we only used nasopharyngeal swab samples as the sputum samples were either inadequate or unavailable.

Figure 2 shows the results of the analysis conducted in patients who tested positive on the COVID-19 PCR test. There were no statistically significant differences in the mean Ct values for E, RdRP, and N genes between patients who tested positive on the RQ test and those who tested negative on the RQ test. However, it should be noted that the analysis was only performed in 123 patients who tested positive on the COVID-19 PCR test, and only 6 of these patients were coinfected with a virus that caused RTI. Further, the absence of statistically significant parameters might be because we did not conduct a subgroup analysis (e.g., sex and age) in the coinfected patients.

Coinfection and superinfection are possible not only with viruses but also with other pathogens, such as bacteria and fungi. A previous study performed a systematic review and meta-analysis on coinfection and superinfection of SARS-CoV-2 and other microbial pathogens.[27] We studied the coinfection of SARS-CoV-2 with other respiratory viruses only because we used the remaining specimens from virus transport media (e.g., UTM), which was obtained to confirm the existence of SARS-CoV-2. In a future study, we plan to use a protocol encircling virus as well as bacteria and fungi to check species, rate, and Ct difference of the pathogens causing coinfection.

In this study, we could confirm that coinfection of SARS-CoV-2 and a respiratory virus did not affect the Ct values of a real-time RT-PCR used for SARS-CoV-2 detection. The coinfection rates observed in this study differed from those reported in previous studies. However, this study had certain limitations. The study was conducted in a single hospital in a single region. To generalize the findings in terms of time and region, a larger number of specimens from multiple centers, collected at different times, should be analyzed. Further, the failure to use various test kits to detect respiratory viruses may have caused statistical bias. In brief, we computed the coinfection rate of SARS-CoV-2 with other RTI viruses and revealed that the mean Ct values for N, RdRP, and E genes in RT-PCR tests were not significantly affected by coinfection.

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CONFLICTS OF INTEREST

There are no potential conflicts of interest relevant to this article.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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