INTRODUCTION

At the end of 2019, a new etiological agent, named later as severe acute respiratory syndrome corona virus-2 (SARS-CoV-2), was identified and has caused more than 8 million infections, and approximately 460,000 deaths to date.\(^1\)\(^2\) Routine confirmation of the disease is based on the detection of SARS-CoV-2 RNA on nasopharyngeal swabs or other respiratory samples by real-time reverse-transcription polymerase chain reaction (rRT-PCR).\(^3\) The duration from onset of symptoms to becoming PCR negative was reported as 4-25 days in a study\(^4\); however, this period may be prolonged up to 4-6 weeks depending on patient characteristics.\(^5\) Previous studies showed that the clinical course and outcomes of patients with prolonged PCR positivity were not different from those who have a shorter duration of PCR positivity. However, these studies had been conducted in relatively small cases series.\(^6\)\(^7\)\(^8\)

The rRT-PCR test also guides the determination of the isolation period in COVID-19 patients. To determine the isolation period,
the Centers for Disease Control and Prevention (CDC) has recommended two different strategies, test-based or symptom-based.9 World Health Organization (WHO) has recommended isolation 10 days after symptom onset, plus at least 3 days after symptoms improved.10 It is not yet known whether it is more advantageous to use a test-based strategy or a symptom-based strategy in discontinuation of isolation. The favourable strategy may change based on countries or centres bounds of capability.5-11 Although the correlation between symptoms and PCR positivity has been mentioned in previous studies, it was not precisely defined.6 Duration of PCR negativity after symptoms improve has not been defined with large case series.

In this study, we aimed to compare patient with and without prolonged PCR positivity and to define the factors that affect long-term PCR positivity. We then aimed to investigate the correlation time between resolution of symptoms and becoming PCR negative in order to determine the optimal isolation periods.

2 | MATERIALS AND METHODS

This study was carried out in Ankara City Hospital, the pandemic reference centre in the capital, between 10 March 2020 and 10 May 2020. Ethical approval was provided by the Turkish Ministry of Health and the Ankara City Hospital Ethical Committee 1. Patients over the age of 18 years, who were hospitalised with the confirmed diagnosis of COVID-19, were included in the study. Patients with positive PCR test for SARS-CoV-2 in the nasopharyngeal swab sample or other respiratory samples were identified as confirmed cases.

The PCR test was performed at the admission to the hospital for all symptomatic or asymptomatic patients. The samples taken were delivered to the laboratory using special transport systems for viruses and following the cold chain rules. Nucleic acid extraction was performed with Bioeksen RNA extraction systems. Bioeksen COVID-19 RT-qPCR Detection Kit was performed with one-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR).

Data were collected retrospectively. Demographic features and clinical findings were recorded from the daily follow-up forms. Age, gender, epidemiological history, comorbid disease, immunosuppression, severity of disease, presence and duration of symptoms were questioned. Laboratory test results were obtained from hospital electronic records. Complete blood count, urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), C-reactive protein, procalcitonin, interleukin 6 (IL-6), ferritin, D-dimer, fibrinogen and blood groups were evaluated. Chest X-ray and thorax computed tomography (CT) were performed for all patients upon admission. Radiological findings were recorded as unilateral/bilateral ground-glass opacity, diffuse infiltration, and consolidation and localisation was classified as peripheral, central and diffuse. Severity of the disease was determined according to the WHO guideline as mild disease, pneumonia, severe pneumonia and critical disease.10 The development of complications was recorded in patient forms. The patients were divided into two groups according to the duration of becoming PCR negative after symptom onset, 0-14 days and ≥15 days. The groups were compared in terms of demographic characteristics, clinical symptoms and signs, laboratory and radiological findings and clinical outcomes including requirement of intensive care unit (ICU), mechanical ventilation and death.

The patients were followed-up based on test-based strategy to decide the isolation period. The sequential sampling was performed in patients whose SARS-CoV-2 PCR was positive in the nasopharyngeal swab or other respiratory samples. The patients were followed-up in hospital until two consecutive negative tests for SARS-CoV-2 were obtained within 24-hour intervals. To demonstrate that the PCR test became negative during follow-up, the first sample was performed routinely 4 or 5 days after the diagnostic PCR test. If negativity was achieved in the first sample, the second sample was taken after 24-36 hours. If otherwise, sampling continued until two consecutive negative samples were obtained. When two consecutive negative PCR test results were provided, the time of the first negative test was considered as the time to turn negative.

Patients were evaluated for the following periods: the duration of symptoms following admission to the hospital, the total duration of symptoms, and the duration of becoming PCR negative after symptom onset.

2.1 | Statistical analysis

SPSS 25.0 (IBM Inc) software system was used for the analyses in the study. Descriptive statistics were presented using mean ± standard deviation and median (IQR: 25th-75th percentile) for continuous data, and frequency and percent for categorical data. The comparisons of continuous and categorical data between independent groups were performed using Student t-test (Mann-Whitney U test in non-parametric conditions) and Chi-square test (Fisher’s exact test

What’s known

In COVID-19 disease, patients who have had prolonged PCR positivity were not different from those who have a shorter duration of PCR positivity according to clinical and demographical features. And it has been known that PCR negativity period after symptom onset changes less than 1 week to 6 weeks.

What’s new

In this study, it was revealed that prolonged PCR positivity did not necessitate additional intervention in the clinical follow-up and treatment of the patients with COVID-19. And, PCR negativity period after symptom onset was reported with the highest number of cases compared with available current literature data.
in non-parametric conditions), respectively. Logistic regression analysis was used for determining the independent factor. Spearman’s non-parametric correlation analysis was used for correlation of time variables. A $P < .05$ was considered as statistically significant.

3 | RESULTS

A total of 339 patients with confirmed COVID-19 infection were included in the study. The mean age was 46.2 years, and 54.3% were males. Of the patients, 34% had at least one comorbidity; hypertension (17.4%) was the most frequent. Of the cases, 90.3% were symptomatic at admission, and cough (55.8%) and fever (43.1%) were the most common symptoms. 9.7% ($n = 33$) patients were tested for having contact with COVID-19 patients without any symptoms. In univariate analysis, there was no significant difference between groups in terms of age, sex, clinical symptoms and signs between groups. The duration of symptoms was longer in patients with prolonged PCR positivity. The baseline demographic, clinical and radiological characteristics of the patients based on the duration of negative PCR test results are presented in Table 1. Consolidation on thorax CT was higher in cases with prolonged PCR positivity ($P = .03$). There was no difference between patients with PCR positivity longer than 14 days and those who had PCR positivity shorter than 14 days in terms of disease severity, clinical deterioration and mortality rates (Table 1).

Laboratory and radiological test results were compared based on the first day of hospital admission (Table 2). Albumin levels were significantly lower in patients with prolonged PCR positivity (42.5 ± 4.5 vs 44.2 ± 4.1, $P = .03$). In multivariate analysis, age ($P = 0.035$) and the duration of symptoms on admission ($P < .001$) were found as independent factors for prolonged PCR positivity (Table 3).

The median duration of symptom at admission was 3 days (IQR: 2-5). Symptoms disappeared at median 7 days (IQR: 5-11) for all patients ($n = 339$) included in the study. The duration of becoming PCR negative after symptom onset was 9 days (IQR: 7-12) (Figure 1). The conversion of the PCR test to negative occurred 3 days (IQR: 2-5) after resolution of symptoms. PCR test was positive for more than 14 days after resolution of symptoms in only six (1.8%) patients. We detected the first control PCR test performed 4-5 days after the positive test as negative in 59% (199) of patients. The patients were hospitalised for a median of 10 days (IQR: 7-13) and an average of 3.3 ± 1.6 tests were performed per patient (Table 1).

Although no significant correlation was detected between symptom duration and the duration of becoming PCR negative after symptom onset ($r = 0.09$, $P = 0.107$), there was a modest trend between these parameters. The symptom duration was prolonged in patients who had longer PCR positivity time (Figure 2).

4 | DISCUSSION

From the beginning of the COVID-19 pandemic, it has been known that some patients have a longer duration of PCR positivity than others. The issue of “ongoing PCR positivity” has significance in two aspects, its effects on disease severity and determining of isolation time (quarantine on asymptomatic). To date, no precise cut-off time has been determined for “prolonged positivity time”. In this study, we accept a longer duration of PCR positivity than 14 days from symptom onset as the cut-off day. That is determined according to guideline recommendations for the isolation period after symptom onset.

We investigated whether there is a difference between groups with and without prolonged PCR positivity and the factor affecting prolonged PCR positivity. There were no differences in terms of severity of infection and clinical outcomes. Prolonged PCR positivity had no additional detrimental effect on the patient’s clinical course. Our results have supported previous studies in the literature that evaluated a smaller number of patients and found no difference between groups. In our study, age and the duration of symptoms on admission were found as the only predictor for prolonged PCR positivity. Similarly, older age was reported to be related to prolonged SARS-CoV-2 positivity in previous studies. Qi et al, who evaluated 147 patients, reported that there was no difference in terms of age between patients with prolonged viral shedding and those who had shorter PCR positivity. However, these patients were reported to have longer duration of symptoms (median: 6 [3-10]) when hospitalised. Therefore, the late hospitalisation of patients may affect the results of the study. As a result, we can say that in light of the results of our study and previous reports, the duration of PCR positivity does not predict or guide clinical course and outcomes of COVID-19.

The second and vital issue with long-term PCR positivity is to determine the termination time of isolation. Despite large amounts of accumulated data, there are issues that are not fully explained in relation to COVID-19. Contagiousness and therefore the isolation period are among them. To decide the isolation period, two strategies are recommended based on the characteristic of patients or centres. Both of them have some advantages and disadvantages. The value of using the test-based strategy in determining the quarantine period is uncertain and even inappropriate according to some reports. The test-based strategy may lead to unnecessarily long durations of isolation, prolonged hospitalisation and overuse of personal protective equipment. On the other hand, there is insufficient evidence for the reliability and feasibility of the symptom-based strategy. As the European Centre for Disease Prevention and Control (ECDC) suggests, if testing and hospital bed capacity allows, isolation period should be ended after 8 days of symptom onset and providing two negative rRT-PCR tests from respiratory samples at 24-hour intervals. If testing capacity is limited, isolation can be terminated at least 3 days after symptoms have improved, or after 8-14 days after symptom onset.

We followed-up patients with test-based strategy, meaning patients were hospitalised until two consecutive negative PCR tests. The total length of hospital stays in our study (median 10 days, [IQR: 7-13]) was similar to the centres that preferred the test-based strategy to determine the isolation period and the patient’s discharge decision. Median length of hospital stay was reported as 13 (IQR:
11.0-17.5) and 19 days (IQR: 16-25) in two previous studies conducted by Qi et al.6 and Hu et al.4, respectively. For the patients with mild to moderate symptoms, these periods are unnecessarily long to stay in hospital, especially in countries with limited sources. If patients are isolated in their homes or other outpatient centres, hospital beds will not be used unnecessarily.

### TABLE 1  
Demographic and clinical characteristics of patients based on the duration of negative PCR test results after symptom onset

| Days of first negative PCR result test after symptom onset | 0-14 days (n = 289) | ≥15 days (n = 50) | P value |
|----------------------------------------------------------|---------------------|------------------|--------|
| Median (IQR)                                             | Median (IQR)        |                  |        |
| Age (y) (median [min-max])                               | 45 [18-93]          | 45 [19-86]       | .16    |
| Symptoms duration on admission                           | 3 (1-4)             | 7 (3-10)         | <.001  |
| Total symptom duration                                   | 7 (5-11)            | 7 (4-10)         | .77    |
| Duration of hospitalisation                              | 10 (8-13)           | 9 (7-12)         | .20    |
| Sex, male                                                | 160 (55.4)          | 24 (48)          | .34    |
| Presence of comorbidity                                  | 96 (33.4)           | 17 (34)          | .94    |
| Hypertension                                             | 49 (17)             | 10 (20)          | .60    |
| Diabetes mellitus                                        | 29 (10)             | 6 (12)           | .67    |
| Chronic lung disease                                     | 13 (4.5)            | 2 (4)            | 1.00   |
| Immunosuppression/malignity                              | 10 (3.5)            | —                | .36    |
| Smoker/Ex-smoker                                         | 49 (17)             | 13 (26)          | .21    |
| Presence of symptoms                                     | 260 (90)            | 46 (92)          | .80    |
| Fever (>37.8 °C)                                         | 125 (43.3)          | 21 (42)          | .87    |
| Coughing                                                 | 158 (54.7)          | 31 (62)          | .34    |
| Sputum                                                   | 7 (2.4)             | 2 (4)            | .63    |
| Dyspnoea                                                 | 64 (22.1)           | 13 (26)          | .55    |
| Sore throat                                              | 58 (20.1)           | 11 (22)          | .75    |
| Positive finding in computerised tomography              | 240 (83)            | 43 (86)          | .60    |
| Unilateral ground-glass opacity                          | 60 (20.8)           | 6 (12)           | .15    |
| Bilateral ground-glass opacity                           | 149 (51.6)          | 29 (58)          | .40    |
| Diffuse infiltration                                     | 19 (6.6)            | 4 (8)            | .76    |
| Consolidation                                            | 28 (9.7)            | 10 (20)          | .03    |
| Radiological localisation                                |                     |                  |        |
| Peripheral                                               | 99 (34.3)           | 22 (44)          | .18    |
| Central                                                  | 8 (2.8)             | 2 (4)            | .65    |
| Diffuse                                                  | 49 (17)             | 9 (18)           | .86    |
| Disease severity                                         |                     |                  | .24    |
| Pneumonia                                                | 155 (54.2)          | 23 (46.9)        |        |
| Mild disease                                             | 91 (31.8)           | 14 (28.6)        |        |
| Severe pneumonia (SARI)                                  | 36 (12.6)           | 11 (22.4)        |        |
| Critical disease                                         | 4 (1.4)             | 1 (2)            |        |
| Presence of complication                                 | 19 (6.6)            | 2 (4)            | .137   |
| Clinical response                                        |                     |                  | .14    |
| Responsive                                               | 243 (84.4)          | 39 (78)          |        |
| Non-responsive (ongoing fever, coughing, etc)            | 19 (6.6)            | 2 (4)            |        |
| Clinical deterioration (respiration > 30/min or O2 saturation < 90%) | 26 (9) | 9 (18) | |
| Need for intensive care unit                             | 33 (12.9)           | 10 (21.7)        | .11    |
| Oxygen supplementation                                   | 75 (26)             | 16 (32)          | .37    |
| Clinical outcome, death                                  | 11 (3.8)            | 1 (2)            | 1.00   |

Bold values are statistical significant.
We found the median duration of first negative PCR test after symptom onset as 9 days (IQR: 7-12), and 14% of patients' tests were positive for SARS-CoV-2 longer than 14 days. Previous studies have reported that PCR positivity usually lasts for up to 3 weeks but can continue for up to 6 weeks.\textsuperscript{5,8} Similar to our study, Ling Y.et al reported the median time as 9.5 [6-11] days in 66 patients.\textsuperscript{13} Qi et al\textsuperscript{6} reported the median duration of viral shedding as 17 days [IQR: 12-21] in 147 patients, longer than our study. However, the study protocol does not seem suitable for giving information about viral shedding as the researchers reported performing the PCR test 1 day after the resolution of symptoms, not before. In our study population, PCR negativity was achieved in the first control PCR test in approximately two-thirds (59%) of patients. The discrepancy between our study and the previous studies that reported longer duration for PCR positivity may result from the methodology of the studies or genetic diversity between the study population.

In test-based strategy, isolation may be discontinued in patients whose fever, cough and dyspnoea disappeared and have a total of two negative respiratory samples for SARS-CoV-2.\textsuperscript{14} In the symptom-based strategy, isolation can be discontinued 10 days after symptom onset and 3 days after disappearance of fever or respiratory symptoms.\textsuperscript{3,14} There is no strong recommendation for determining isolation periods. The recommendations depend on the studies with limited cases.\textsuperscript{12,15} Well-planned studies are needed to determine the isolation period, but with the available literature, isolation can be suggested for up to 14 days after the onset of symptoms.\textsuperscript{16} Hu et al\textsuperscript{4} used test-based strategy for the decision to discharge patients from hospitals, and they have reported that the median duration of negative conversion of PCR test was 14 days (IQR: 10-18). Ai Tang Xiao et al suggested repeated confirmation of RT-PCR test from respiratory specimens for safe discharges and discontinuation of isolation regardless of duration.\textsuperscript{8}

| Laboratory test | All patients (n = 339) | Day of first negative PCR result test after symptom onset | P value |
|-----------------|----------------------|--------------------------------------------------------|---------|
|                 | Median [min-max]     | 0-14 days (n = 289) | ≥15 days (n = 50) |         |
| White blood cell count - x10^9/L | 5255 [120-59290] | 5205 [120-59290] | 5435 [2920-18760] | .47 |
| Neutrophil count - x10^9/L | 3270 [200-16460] | 3280 [200-13410] | 3225 [1430-16460] | .84 |
| Lymphocyte count - x10^9/L | 1180 [150-7360] | 1170 [150-7360] | 1265 [330-3090] | .49 |
| Monocyte count - x10^9/L | 330 [40-1460] | 330 [40-1460] | 330 [130-670] | .79 |
| Haemoglobin - g/L | 13.8 [7.1-17] | 13.8 [7.1-17] | 13.8 [10.9-16.4] | .65 |
| Platelet count - x10^9/L | 206 500 [13000-436000] | 209 000 [13000-436000] | 198 000 [14500-406000] | .40 |
| Urea | 26 [11-52] | 27 [11-52] | 25 [13-45] | .55 |
| Creatinine - μmol/L | 0.8 [0.1-46753] | 0.8 [0.1-46753] | 0.8 [0.5-43831] | .75 |
| AST, U/L | 24 [7-500] | 24 [7-500] | 26 [11-74] | .78 |
| ALT, U/L | 28 [7-634] | 28 [7-634] | 27 [9-113] | .96 |
| Albumin - g/L | 45 [30-54] | 45 [33-54] | 44 [30-50] | .03 |
| Creatine Kinase - μ/L | 93 [12-5395] | 93.5 [12-5395] | 93 [20-860] | .71 |
| LDH U/L | 218 [106-697] | 218 [106-697] | 211 [160-646] | .77 |
| C-reactive protein - mg/L | 0.009 [0.00007-0.9] | 0.009 [0.00007-0.9] | 0.009 [0.0001-0.3] | .35 |
| Procalcitonin (PCT) μg/L | 0 [0-2] | 0.03 [0-1.3] | 0.03 [0-2] | .84 |
| Ferritin, μg/L | 118.5 [1-1566] | 118 [1-1023] | 178 [10-1566] | .30 |
| D-dimer - μg/L | 0.4 [0-35.2] | 0.4 [0-35.2] | 0.4 [0-2] | 1.00 |
| Fibrinogen - g/L | 3.1 [1.3-291] | 3 [1.3-291] | 3.2 [2-8.5] | .28 |
| IL-6, pg/mL | 12.3 [2-289] | 11.3 [2-289] | 24.5 [4.3-80] | .13 |

Bold values are statistical significant

| Laboratory test | Multivariate analysis |
|-----------------|----------------------|
|                 | OR 95% CI             | P value |
| Age (y)         | 1.022 1.002-1.042     | .035    |
| Sex (male)      | 0.783 0.385-1.593     | .500    |
| Symptoms duration on admission | 1.456 1.306-1.624 | <.001 |
| Total symptom duration | 1.101 0.994-1.220 | .065 |
| Consolidation   | 0.580 0.219-1.532     | .271    |
| Albumin - g/L  | 0.899 0.807-1.003     | .056    |

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We found duration of symptoms as an independent factor for prolonged PCR positivity. In addition, there was a modest trend between the duration of symptoms and the duration of becoming PCR negative. The duration of PCR positivity prolonged as the symptom duration increased. PCR test became negative at median 3 days (IQR: 2-5) after resolution of symptoms. The test-based strategy has
some difficulties in clinical practice, and possibly leads to a longer isolation period than recommended. According to our study results, symptoms persisted for 7 days (IQR: 5-11) with PCR testing positive for an additional 3 days (IQR: 2-5) after symptom resolution. As such, the recommendations of the WHO and ECDC regarding discontinuation of isolation seems an optimal approach. Based on the study results, we suggest that symptom-based strategy may be used to decide isolation discontinuation, especially in patients who can isolate themselves until 10-14 days after symptom onset in their homes or outpatients’ institutions, or in centres with limited bed or test capacity.

There are limitations in our study. Because of the retrospective characteristic of the study and the presence of a large number of patients admitted to our hospitals, we could not perform PCR testing every other day. If it was possible, the results might be that the time to turn PCR negative could be shorter.

In this study, we investigated the impact of ongoing PCR positivity on patients’ outcomes and the time correlation between the resolution of symptoms and becoming PCR negative. Our study supported the previous study results reporting that prolonged PCR positivity had no detrimental effect on patients’ outcomes. In addition, we think that our study will provide a contribution to the literature in determining the isolation time. According to our study results, viral shedding has been ending 3 days (IQR: 2-5) after symptom resolution. When testing cannot be performed or the symptom-based strategy is used, it seems to be sufficient to continue isolation for a total of 10-14 days after symptom onset (2-5 days after symptoms regress).

DISCLOSURES
The authors declare no disclosures.

AUTHOR CONTRIBUTIONS
Concept/design, Data analysis/interpretation and Critical revision of article: Fatma Eser, Bircan Kayaaslan and Rahmet Güner. Statistics: Fatma Eser and Bircan Kayaaslan. Data collection: Fatma Eser, Bircan Kayaaslan, Rahmet Güner, Imran Hasanoğlu, Ayşe Kaya Kalem, Adalet Aypak and Esragül Akıncı.

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