

# Accuracy of RT-PCR Test in diagnosing COVID-19: A retrospective Study

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## ABSTRACT

**Background:** The aim of this study was to compare the accuracy of a real-time reverse transcription polymerase chain reaction (RT-PCR) test in severe and critical cases of covid-19 if computerized tomography scan (CT) scan was considered as a reference test.

**Methods:** This retrospective study was conducted on hospitalized patients in the referral hospital of covid-19 in the southeast of Fars province in Iran. The criteria for critical cases were as follows: invasive mechanical ventilation, admission to intensive care unit (ICU), or death. Also, those patients who needed oxygen support were categorized as severe cases. Sensitivity, specificity, and predictive values were estimated to compare the accuracy of RT-PCR test in severe and critical cases.

**Results:** This study described the higher accuracy (72.8 vs 65.6), sensitivity (73.2 vs 63.4), specificity (69.9 vs 65.2), and negative predictive values (28 vs 15.9) of RT-PCR test in severe cases and critical cases, respectively. On the other hand, the positive predictive value of RT-PCR test was higher among critical cases than severe cases (99.4 vs 94.2, respectively).

**Conclusion:** The accuracy of RT-PCR test, if CT scan is considered as a reference test, was lower in critical cases than in severe cases of covid-19. Therefore, the false negative of RT-PCR test if the CT scan is positive can predict lower prognosis in covid-19 patients.

**Keywords:** Accuracy, Sensitivity, Specificity, predictive value, RT-PCR test, Covid-19

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## INTRODUCTION

Covid-19 was first reported in Wuhan, China, in early 2020, and subsequently spread worldwide. The World Health Organization (WHO) declared a public health emergency in January 2020<sup>2,1</sup>. The first cases of covid-19 were detected in Iran on February 18, 2020. The widespread outbreak of covid-19 poses significant threats to the global economy and health care system<sup>4</sup>. The disease is highly contagious, and in severe cases, it can lead to lung involvement and cause the acute respiratory distress syndrome (ARDS) or failure of other organs<sup>5</sup>. The most common clinical symptoms, in addition to fever and cough, are shortness of breath, headache, muscle aches, and fatigue<sup>6</sup>. According to the World Health Organization, patients are divided into 4 groups: asymptomatic, mild, severe and critical, based on the severity of symptoms. Studies showed that about 15% of patients were severe and need oxygen therapy and 5% were critical form<sup>7</sup>.

The WHO has introduced the real-time reverse transcription polymerase chain reaction (RT-PCR) test as a "gold standard test" for detecting covid-19<sup>8</sup>. However, the sensitivity of this laboratory test may vary depending on the quality of the kit, the skill of the sampler, the time of preparation of the sample, and the manner of storage and transfer of the sample, the sampling conditions, and it is reported between 60% and 91%<sup>9</sup>. On the other hand, diagnosis based on this method is time-consuming, nonetheless, the use of computerized tomography (CT) scan in rapid diagnosis and clinical decision-making has become highly important<sup>10</sup>. In patients with suspected negative RT-PCR, physicians recommend a CT scan. The CT scan is a method that is more sensitive to RT-PCR at certain stages of the disease. According to the results of

various studies, the sensitivity of CT scan is more than 90% and its specificity is about 60% or lower<sup>11,12</sup>. Based on the meta-analysis, the sensitivity of CT scan (95%) is higher than the first RT-PCR test (91%).<sup>13</sup> Studies have shown that patients with negative RT-PCR test and positive CT scan eventually were found to have covid-19 and a positive RT-PCR test after repeated swabbed tests.<sup>14</sup> Due to the low specificity of CT scan, the American Radiological Association recommends that CT scan not be used as a screening tool or as the first method to diagnose covid-19, instead CT scan be used more in hospitalized and symptomatic patients<sup>15</sup>. In patients with covid-19, the CT scan usually becomes positive through 2 to 3 days after the onset of symptoms<sup>13</sup>.

Considering the limited studies on the difference in sensitivity of RT-PCR test based on the severity of clinical symptoms, the present study was conducted to compare the sensitivity of RT-PCR test in severe and critical hospitalized patients by considering CT scan results of patients as a reference diagnostic test.

## METHODS

**Study Design and Participants:** This retrospective study was conducted on hospitalized patients in the referral hospital of covid-19 in the southeast of Fars province in Iran. The inclusion criteria were suspected patients for covid-19 who underwent high resolution chest CT and real-time RT-PCR between February 19 and November 10, 2020. Exclusion criteria were incomplete clinical or laboratory information and chest CT images. In this study the patients were divided in to 2 groups: severe cases and critical cases. The criteria for critical cases included invasive mechanical ventilation, admission to intensive care

unit (ICU), or death. Those patients who needed for oxygen support were categorized as severe cases. The demographic and clinical information (initial clinical symptoms at the time of hospital admission, comorbidities) were extracted from electronic records of hospitalized cases. The RT-PCR of respiratory section specimens from nasopharyngeal swab, oropharyngeal swab, endotracheal aspirate, or bronchoalveolar lavage were performed using real-time RT-PCR kits with National Medical Products Administration Emergency approval. All procedures were done by following the instructions of the ethic committee of Jahrom University of Medical Sciences (approval No. IR.JUMS.REC.1399.001). Signed informed consent was exempted due to the retrospective nature of the study.

**CT Image Acquisition and Analysis:** After the swab sampling or before admission to the hospital, noncontrast high resolution CT thorax images were acquired with 1 mm slice thickness, following the acquisition of parameters of the usual protocol, then they were reformatted with soft tissue and lung windows. All images were transferred to a stand-alone work station for analysis. Typical chest CT findings were extracted from previously published reports and served as diagnostic reference including ground-glass opacification with or without consolidation, crazy paving pattern, peripheral and diffuse distribution, and bilateral/multilobular involvement.<sup>16,17</sup> Two experienced general radiologists who had specific trainings regarding covid-19 presentation on chest CT by online courses and real-life cases independently reviewed the images to determine whether CT findings were positive. Image readers were aware of the patients' epidemiologic history and clinical characteristic, but were blind to personal information and RT-PCR results, which is the same as clinical setting. At first, their own results were recorded for interobserver reliability test. Then, the final CT results were determined by their consensus discussion for diagnostic performance analysis.

**Statistical Analysis:** The categorical variables were reported as frequency and percentages. Pearson's chi square or Fisher exact tests were used to compare the difference of proportions. Using CT scan as a diagnostic reference test, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of initial RT-PCR test were calculated with 95% confidence interval (CI) in severe and critical hospitalized patients. Statistical analysis was performed using STATA version16 (STATACorp). A  $P < .05$  indicated a significant difference.

The hospitalized suspected cases of covid-19 were 4226 patients during February 19 and December 31, 2020. 1 out of 3 patients ( $n = 1518$ ) were excluded from the analysis due to incomplete laboratory information or chest CT images. The analysis was done on 2079 hospitalized cases. This study showed that the false negative RT-PCR test is not different among male and female patients; however, the probability of false negative was higher among those older than 70 years, especially in critical cases (48.1%) than in severe (33.1%) cases ( $P < .001$ ). The probability of false negative RT-PCR test in severe cases was significantly higher than critical cases with initial symptoms including cough (42.4 vs 24.5), fever (36.3 vs 20.7), myalgia (28.9 vs 6.6), headache (5.5 vs 0.9), and dizziness (5.8 vs 0.9), respectively ( $P < .05$ ). On the other hand, the false negative of RT-PCR among critical cases were higher than in severe patients with initial symptoms, including loss of consciousness (45.3 vs 8.7) PO<sub>2</sub> < 93% (59.4 vs 33.8), and chest pain (4.7 vs 4.2), respectively ( $P < .05$ ) (Table 1).

Comorbidities such as cancer (11.3 vs 3.5), diabetes (23.6 vs 16.7), chronic heart disease (44.3 vs 23.8), blood disorder (2.8 vs 0.6), and kidney failure (3.8 vs 2.3) increased the probability of false negative RT-PCR in critical cases more than in severe patients. However, the false negative of RT-PCR among severe cases was higher than in critical cases in patients with immune disorders (1.6 vs 0.3) and nervous disorders (4.8 vs 3.8) ( $P < .05$ ) (Table1).

**Factors Affecting the Accuracy of RT-PCR Test:** According to the results of univariate and multivariate analysis, the clinical symptoms that significantly decreased the accuracy of RT-PCR test were the loss of consciousness (OR = 0.47 [0.35, 0.64] & OR = 0.67 [0.47, 0.95]), chest pain (OR = 0.69 [0.52, 0.93] & OR = 0.65 [0.47, 0.9]), respectively ( $P < .05$ ). The univariate and multivariate analysis for comorbidities showed that hypertension (OR =1.27 [1.06, 1.52] & OR = 1.29 [1.03, 1.61]) had a significant increasing effect on the accuracy of RT-PCR test (Table 2).

**The Performance of RT-PCR Test in Severe and Critical Patients:** The results of the study described the higher accuracy (72.8 vs 65.6), sensitivity (73.2 vs 63.4), specificity (69.9 vs 65.2), and negative predictive value (28 vs 15.9) of RT-PCR test in severe cases than critical cases, respectively. On the other hand, the positive predictive value of PCR-test was higher among critical cases than severe cases (99.4 vs 94.2), respectively (Table 2).

## RESULTS

Table1. Compared Demographic and Clinical Characteristics of Severe and Critical Patients by RT-PCR Test Results in Positive CT Cases, Iran, Southeast of Fars Province, 2020<sup>a</sup>

Variables		Severe			Critical			P Value
		TP* (1527)	FN** (560)	Total (2398)	TP* (184)	FN** (106)	Total (311)	
Sex	Female	691 (45.2)	236 (42.1)	1065 (44.4)	59 (32.1)	46 (43.4)	105 (36.2)	.5
	Male	837 (54.8)	324 (57.9)	1333 (55.6)	125 (67.9)	60 (56.6)	185 (63.8)	
Age group	0-19	38 (2.5)	24 (4.2)	70 (2.9)	0 (0)	5 (4.7)	5 (1.7)	<.001
	20-49	711 (46.5)	171 (30.6)	1017 (42.4)	27 (14.7)	19 (17.9)	46 (15.9)	
	50-69	542 (35.5)	180 (32.2)	830 (34.6)	71 (38.6)	31 (29.3)	102 (35.2)	
	70+	238 (15.6)	185 (33.1)	487 (20.3)	86 (46.7)	51 (48.1)	137 (47.2)	
Fever		811 (53.1)	203 (36.3)	1199 (50)	88 (47.8)	22 (20.7)	110	<.001
Cough		908 (59.4)	237 (42.4)	1314 (54.8)	100 (54.4)	26 (24.5)	126 (43.4)	<.001
Myalgia		833 (54.5)	162 (28.9)	1144 (47.7)	78 (42.4)	7 (6.6)	85 (29.3)	<.001

Dyspnea	938 (61.4)	286 (51.1)	1405 (58.6)	138 (75)	66 (62.3)	204 (70.3)	<.001
Loss of consciousness	31 (2)	49 (8.7)	91 (3.8)	27 (14.7)	48 (45.3)	75 (25.9)	<.001
Headache	219 (14.3)	31 (5.5)	285 (11.9)	15 (8.2)	1 (0.9)	16 (15.5)	<.001
Dizziness	142 (9.3)	32 (5.8)	201 (8.4)	13 (7.1)	1 (0.9)	14 (4.8)	.003
Chest pain	110 (7.2)	24 (4.2)	165 (6.9)	12 (6.5)	5 (4.7)	17 (5.9)	.04
PO2<93%	590 (38.6)	189 (33.8)	894 (37.3)	138 (75)	63 (59.4)	201 (69.3)	<.001
Gastrointestinal symptoms***	270 (17.7)	77 (13.8)	398 (16.6)	21 (11.4)	8 (7.6)	29 (10)	.2
<b>Comorbidities</b>							
Cancer	21 (1.4)	20 (3.5)	46 (1.9)	11 (5.9)	12 (11.3)	23 (7.9)	.001
Liver disease	61 (4)	7 (1.3)	14 (0.6)	2 (1.1)	1 (0.9)	3 (1.03)	.3
Diabetes	286 (18.7)	94 (16.7)	436 (18.2)	62 (33.7)	25 (23.6)	87 (30)	.002
Blood disorders	14 (0.9)	3 (0.6)	22 (0.9)	3 (1.63)	3 (2.83)	6 (2.1)	.01
Immune disorders	11 (0.7)	9 (1.6)	46 (1.9)	0 (0)	1 (0.9)	1 (0.3)	.03
Chronic heart disease	203 (13.3)	133 (23.8)	386 (16.1)	53 (28.8)	47 (44.3)	100 (34.5)	<.001
Kidney failure	17 (1.1)	13 (2.3)	34 (1.4)	6 (3.3)	4 (3.8)	10 (3.5)	<.001
Nervous disorder	18 (1.2)	27 (4.8)	53 (2.2)	6 (3.3)	4 (3.8)	10 (35)	.004
Hypertension	380 (24.9)	153 (27.3)	611 (25.5)	76 (41.3)	33 (31.1)	109 (37.6)	.1
Chronic respiratory disease	38 (2.5)	25 (4.5)	72 (3.02)	8 (4.4)	3 (2.8)	11 (3.8)	.3

\*True Positive, \*\*False Negative, \*\*\* Includes abdominal pain, anorexia, diarrhea, nausea, or vomiting.

°RT-PCR test, real-time reverse transcription polymerase chain reaction; CT, computerized tomography.

Table 2. The Demographic and Clinical Features Affecting the Accuracy RT-PCR Test Among Critical and Sever Cases of Covid-19, Iran, Southeast of Fars Province, 2020

Variables	Univariable OR (95% CI)	P Value	Multivariable OR (95% CI)	P Value
<b>Demographic covariates</b>				
Age				
0-19	-	-	-	-
20-49	0.41(0.27,0.64)	<.001	0.31 (0.186, 0.53)	<.001
50-69	1.36 (1.1,1.66)	.003	0.96 (0.73, 1.26)	.78
70+	1.3 (1.1, 1.62)	.009	0.97 (0.76, 1.25)	.83
Sex (female/male)	1.21 (1.03,1.42)	.02	1.32 (1.1, 1.59)	.003
<b>Symptoms at initial evaluation</b>				
Fever	1.94 (1.65,2.27)	<.001	1.74 (1.43, 2.11)	<.001
Cough	1.86 (1.62, 2.22)	<.001	1.74 (1.44, 2.1)	<.001
Myalgia	2.35 (1.99,2.76)	<.001	1.87 (1.54, 2.28)	<.001
Dyspnea	1.59 (1.87, 2.18)	<.001	1.76 (1.46, 2.13)	<.001
Loss of consciousness	0.47 (0.35, 0.64)	<.001	0.67 (0.47, 0.95)	.023
Headache	1.56 (1.19,2.04)	.001	1.36 (1, 1.85)	.05
Chest pain	0.69 (0.52, 0.93)	.01	0.65 (0.47, 0.9)	.01
PO2<93%	1.74 (1.48, 2.04)	<.001	1.61 (1.32, 1.96)	<.001
Gastrointestinal symptoms***	1.22 (0.99, 1.51)	.07	-	-
<b>Underlying conditions</b>				
cancer	-	-	-	-
Liver disease	0.48 (0.19, 1.24)	.13	-	-
Diabetes	1.66 (0.957, 1.42)	.13	-	-
Blood disorders	0.37 (0.19, 0.74)	.005	0.41 (0.19, 0.87)	.02
Immune disorders	0.75 (0.34, 1.67)	.48	-	-
Chronic heart disease	0.63 (0.52, 0.77)	<.001	0.61 (0.479, 0.77)	<.001
Kidney failure	0.41 (0.27, 0.62)	<.001	0.43 (0.27, 0.68)	<.001
Nervous disorder	-	-	-	-
Hypertension	1.27 (1.06, 1.52)	.009	1.29 (1.03, 1.61)	.03
Chronic respiratory disease	0.84 (0.54, 1.30)	.43	-	-

\*\*\* Includes abdominal pain, anorexia, diarrhea, nausea or vomiting.

Table 3. Compared the Diagnostic Performance of RT-PCR Among Severe and Critical Patients in COVID-19, Iran, Southeast of Fars Province, 2020

	Results				Test Performance				
	TP <sup>a</sup> (N)	FP <sup>b</sup> (N)	TN <sup>c</sup> (N)	FN <sup>d</sup> (N)	Sensitivity % (95% CI)	Specificity% (95% CI)	Positive Predictive Value% (95% CI)	Negative Predictive Value% (95% CI)	Accuracy% (95% CI)
Overall	1711	95	237	666	71.2 (71.1, 71.3)	72.7 (72.5, 72.9)	95.1 (95, 95.2)	25.3 (25.2, 25.4)	71.4 (71.3, 71.4)
sever	1527	94	217	560	73.2 (73.1, 73.3)	69.9 (69.7, 70.2)	94.2 (94.1, 94.3)	28 (27.9, 28.1)	72.8 (72.7, 72.8)
critical	184	1	20	106	63.4 (63.3, 63.6)	95.2 (94.9, 95.5)	99.4 (99.4, 99.5)	15.9 (15.7, 16.1)	65.6 (65.4, 65.7)

<sup>a</sup> True positive; <sup>b</sup> False positive; <sup>c</sup> True negative; <sup>d</sup> False negative.

## DISCUSSION

This study indicated the sensitivity of RT-PCR test to be 71.2% (71.1, 71.3), the specificity 95.1% (95, 95.2), and accuracy 71.4% (71.3, 71.4) among hospitalized patients if CT scan was considered as a diagnostic reference test. While the results of the subgroup analysis showed higher sensitivity (73.2 [95% CI, 73.1, 73.3] vs 63.4 [95%CI, 63.3, 63.6]), specificity (69.9 [69.7, 70.2] vs 95.2 [94.9, 95.5]), and accuracy (72.8 [72.7, 72.8] vs 65.6 [65.4, 65.7]) of RT-

PCR test in severe cases than in critical cases, respectively. A study done in China that considered CT scan as a reference test, found an RT-PCR sensitivity of 65%, specificity of 83%, and accuracy of 67%<sup>14</sup>. The study of Cheng and one Italian study reported that the sensitivity of RT-PCR was about 50% and the specificity was 100% if the CT scan was used as a reference<sup>18,19</sup>. The studies reported that the sensitivity of RT-PCR test depends on the inherent performance of the testing kit, the experience of sampler, sampling volume, the condition during transport,

storage samples, laboratory practice, the interval since acquiring infection, and sample collection; therefore, it may be the artificial variability in the value of the accuracy of RT-PCR between studies<sup>9</sup>. On the other hand, the higher accuracy of RT-PCR test in severe cases than in critical may be affected by the segment long lobes or the rate of long involvement. The hypothesis that may justify this dissimilarity is the difference between the time of illness and the time of sampling in the group with severe form and the group with critical form of the disease; however, in the present study, this information was not available.

Our study showed that the sensitivity and accuracy of RT-PCR test depend on some clinical symptoms, including loss of consciousness and chest pain, which had negative effects on the accuracy of RT-PCR test. However, a study in China on 33 patients reported a nonsignificant association between clinical features and the sensitivity of RT-PCR test, with the exception of leukocyte and platelet counts. The nonsignificant association in the Chinese study may be due to the low sample size.<sup>18</sup> The early diagnosis of covid-19, especially in critical cases, may have an important role in improving its prognosis. Due to the lower sensitivity of RT-PCR test, especially in critical cases, we should emphasize that CT scan was used as a complementary method for covid-19 diagnosis, especially in hospitalized patients. This finding is consistent with the recommendations of the American College of Radiology<sup>16</sup>.

Our study results, showed aligned with the literature, the accuracy of RT-PCR test did not differ according to sex, but the sensitivity was lower in older cases.<sup>20,21</sup> Some studies reported a lower sensitivity of RT-PCR test in the elderly may be due to the sampling error caused by the difficulties of sampling among the elderly groups<sup>20</sup>.

Given the fact that the positive predictive value in situations where the prevalence of the disease is high, hospitalization rates are also higher and that Jahrom is also one of the cities with a high prevalence of the disease, thus, the predictive value of CT scan in this study may be acceptable. The other hand, radiologists reported that the scans have an important role in the diagnostic accuracy of CT scan for detecting covid-19 infection<sup>13</sup> Therefore, the sensitivity and specificity of RT-PCR test reported according to this method may have been over- or underestimated. One of the limitations of the present study was the lack of information on the time between the onset of the disease and RT-PCR testing. Thus, the results of this study should be interpreted with caution.

## CONCLUSION

According to our study, the accuracy of RT-PCR test, if CT scan was considered as a reference test, was lower in critical cases than in severe cases of covid-19. Therefore, the false negative of RT-PCR test, if the scan was positive, could predict lower prognosis in covid-19 patients. Therefore, it is suggested that in future studies, the sensitivity of PCR test in hospitalized patients be evaluated by controlling the effect of the length of time between the onset of symptoms and PCR test sampling.

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**Ethical considerations:** The protocol of this study was approved by the Ethics Committee of Jahrom University of Medical Sciences (Cod: IR.JUMS.REC.1399.001).

**Conflict of interest:** The authors declare that there is no conflict of interest

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