



## Original Research



### **Comparison of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and IgM and IgG antibody test for the diagnosis of SARS-CoV-2 infection**

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#### HIGHLIGHTS

Instead of using the tests as an alternative to each other, using them as complementary will be indispensable for definitive diagnosis.

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#### ABSTRACT

In the present study, we compared the real-time reverse-transcription polymerase chain reaction (RT-PCR) and total IgM-IgG antibody tests for diagnosis SARS-CoV-2 patients. SARS-CoV-2 patients were divided into four additional subgroups according to clinical examination, and Computed Tomography (CT) for SARS-CoV-2. Groups were included 60 mild cases, 111 moderate cases, 53 severe cases and 105 normal cases. In a mild group, 52.5% of 60 cases were found to be male, 45.9% female, and the average age was found as  $38.4 \pm 2.011$ . The positive ratio was found as 80.3% in the RT-PCR test, while 39.3% in total IgM/IgG. In a moderate group, 49.1% of 111 cases were found to be male, 50% female, and the average age was  $45.05 \pm 1.519$ . The positive ratio was found as 85.7% in the RT-PCR test, while 54.5% in total IgM/IgG. In a severe group, 53.7% of 53 cases were found to be male, 44.4% female, and the average age was  $55.5 \pm 2.122$ . The positive ratio was found as 75.9% both in RT-PCR and total IgM/IgG test. In a normal group with no involvement according to Computed Tomography (CT), 49.1% of 105 cases were found to be male, 50% female, and the average age was found as  $34.8 \pm 1.391$ . The positive ratio was found as 95.3% in the RT-PCR test, while 5.7% in total IgM/IgG. Chronic diseases were detected more in severe cases, suggesting that persons who have chronic diseases or decreased immunity, such as diabetes mellitus, cardiovascular diseases, hypertension, and lung disease, are at a higher risk for developing severe COVID-19 if they are infected with SARS-CoV-2. According to our results, we can suggest a higher detection sensibility in RT-PCR than in total IgM/IgG antibody test for mild, moderate and normal group, while the detection sensibility of IgM/IgG antibody increases in a severe group with diffuse bilateral involvement according to CT.

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## 1. INTRODUCTION

The new coronavirus (SARS-CoV-2) spread rapidly all over the world and caused coronavirus disease (COVID-19) in infected people after its appearance in Wuhan, China, in December 2019. The World Health Organization identified COVID-19 (2019 coronavirus disease) disease. COVID-19 caused an epidemic and led to a major challenge for health systems. For reducing the risk of spread, it should be investigated and developed effective treatment and diagnostic options.<sup>1</sup> The signs and symptoms of SARS-CoV-2 infection are known to be not specific; most are found to be associated with respiratory complications such as cough dyspnea, and viral pneumonia. However, the mortality of critical patients with SARS-CoV-2 pneumonia is also noteworthy.<sup>1,2</sup> For this reason, specific COVID-19 diagnostic tests are necessary in order to confirm suspected cases.<sup>3,4</sup>

In order to diagnose SARS-CoV-2 infection, the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test, which detects the presence of viral RNA, is used. This molecular test is known to be useful in the first three weeks of infection and is currently the WHO recommended reference standard.<sup>5</sup> RT-PCR tests were known to be based on the RNA-dependent RNA polymerase (RdRp) gene of the ORF1ab sequence, E gene, N gene, and S gene of the SARS-CoV-2 genome.<sup>6</sup> Among these tests, RT-PCR analyses targeting the RdRp test has the highest analytical precision.<sup>4</sup>

To determine epidemiological surveillance, the immunological tests can be thought of as supplementary diagnostic aid and important support. These tests are based on the detection of immunoglobulin IgM and IgG seen in the second week of infection against SARS-CoV-2.<sup>5</sup> Rapid serological tests based on the detection of antibodies in venous and capillary blood give results in a few minutes. Nevertheless, the sensitivity of the test depends on the timing when the sample was taken and can be more than 90% since the second week of symptom beginning.<sup>7</sup> The use of these tests may contribute significantly to improve the accuracy of the clinical diagnosis, especially in hospitalized patients with negative molecular test results and in patients who have just undergone RT-PCR.<sup>5,7</sup>

In this study, we aim to evaluate the rapid serological test for detection of IgM and IgG antibodies, by means of comparing its additional diagnostic performance with the one of the RT-PCR, for detecting SARS-CoV-2 infections under field conditions.

## 2. MATERIAL AND METHOD

In this study, 329 patients diagnosed with SARS-CoV-2 in Siirt training and research hospital (Siirt University, Turkey) from March 15th to July 01st, 2020, were included as the case group. To diagnose patients, pneumonia diagnosis protocol for novel coronavirus infection was followed, subjected to the tests including clinical examination, Computed Tomography (CT) and RT-PCR for SARS-CoV-2. SARS-CoV-2 patients were divided into four additional subgroups according to CT results. Groups were included 60 mild cases with unilateral pulmonary involvement (32 males and 28 females, median age were 34 [17-77]), 111 moderate cases with bilateral pulmonary involvement (55 males and 56 females, median age were 43 [19-99]), 53 severe cases with bilateral diffuse involvement (28 males and 24 females, median age was 55 [20-92]) and 105 normal cases with no involvement according to CT (52 males and 53 females, median age was 32 [17-84]).

Data on biochemical parameters were obtained from 329 confirmed SARS-CoV-2 infection patients, validated by a wide range of studies including clinical examination, laboratory tests, and

RT-PCR for SARS-CoV-2, with SARS-CoV-2 RdRpq PCR detection kit (Bioeksen, İstanbul, Turkey), as well as using a SARS-CoV-2 IgM/IgG detection kit (Colloidal Gold, China). Clinical and laboratory information was collected during routine clinical studies, and this study was approved by the Siirt University Non-Interventional Clinical Research Ethics Committee (Decision No: E-5739).

SPSS software version 22.0 was used for statistical analysis. All quantitative data in non-normal or unknown distribution were expressed as median and interquartile range  $p < 0.05$  was defined as statistically significant in all tests.

### 3. RESULTS AND DISCUSSION

In the present study, we compared the real-time reverse-transcription polymerase chain reaction (RT-PCR) and total IgM-IgG antibody tests for diagnosis SARS-CoV-2 patients. SARS-CoV-2 patients were divided into four additional subgroups according to clinical examination, and Computed Tomography (CT) for SARS-CoV-2. Groups were included 60 mild cases (32 males and 28 females, median age were 34 [17-77]), 111 moderate cases (55 males and 56 females, median age were 43 [19-99]), 53 severe cases (28 males and 24 females, median age were 55 [20-92]) and 105 normal cases (52 males and 53 females, median age were 32 [17-84]). Liu et al. 2020 reported that there were 44 moderate cases, 52 severe cases, and 37 critical cases with no significant difference in gender and age among three subgroups. According to our results, there is no significant difference of gender, whereas the age of severe (55.5) and moderate (45.05) group's means higher than mild (38.4) and normal (34.8) group's means.

[Table 1](#) shows the comparison of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and IgM-IgG antibody test for the diagnosis of SARS-CoV-2 infection. In mild group, 52.5% (n=32) of 60 cases were found to be male, 45.9% (n=28) female, and the average age was found as  $38.4 \pm 2.011$ . The positive ratio was found as 80.3% (n=49) in RT-PCR test, while 39.3% (n=24) in total IgM/IgG. In moderate group, 49.1% (n=55) of 111 cases were found to be male, 50% (n=56) female, and the average age was  $45.05 \pm 1.519$ . The positive ratio was found as 85.7% (n=96) in RT-PCR test, while 54.5% (n=61) in total IgM/IgG antibody test. In severe group, 53.7% (n=29) of 53 cases were found to be male, 44.4% (n=24) female and the average age was  $55.5 \pm 2.122$ . The positive ratio was found as 75.9% (n=41) both in the RT-PCR test and total IgM/IgG antibody test. In normal group with no involvement according to CT, 49.1% (n=52) of 105 cases were found to be male, 50% (n=53) female, and the average age was found as  $34.8 \pm 1.391$ . The positive ratio was found as 95.3% (n=101) in the RT-PCR test, while 5.7% (n=6) in IgM/IgG antibody test. According to the results we obtained from this study, we can suggest a higher detection sensibility in RT-PCR than in total IgM/IgG antibody test for mild, moderate and normal group, while the detection sensibility of IgM/IgG antibody increases in a severe group with diffuse bilateral involvement according to CT ([Figure 1](#)).

In recent, Liu et al.,<sup>1</sup> studied in RT-PCR detection for viral RNA in 133 patients the instead of an infected with SARS-CoV-2. They reported the positive ratio was 65.91% in moderate cases, 71.15% in severe cases and 67.57% in critical cases, respectively. In our study, according to RT-PCR detection results for viral RNA in 329 patients infected, the positive ratio was detected as 80.3% in mild cases, 85.7% (n=96) in moderate cases, 75.9% (n=41) in severe cases and 95.3% (n=101) in normal cases (Table 1). In our study, the positive ratio of RT-PCR was higher than in their study. RT-PCR test is known to be useful in the first three weeks of infection and is currently the WHO recommended reference standard.<sup>5</sup> It is known that RT-PCR analyses targeting the RdRp test have the highest analytical precision.<sup>4</sup>

Liu et al.,<sup>1</sup> reported that the positive ratio of IgM/IgG antibody detection in patients was 79.55%/93.18%, 82.69%/100%, and 72.97%/97.30% in moderate, severe, and critical cases, respectively. In this study, we measured the positive ratio of total IgM/IgG antibody in patients. We found the positive ratio of total IgM/IgG antibody as 39.3% (n=24) in mild cases, 54.5% (n=61)

in moderate cases, 75.9%(n=41) in severe cases and 5.7%(n=6) in normal cases. Supplement serum IgM / IgG antibody detection against SARS-CoV-2 internal nucleoprotein (NP) and surface spike protein receptor binding site (RBD) may compensate for RT-PCR deficiencies in some cases.<sup>6,8</sup> After infection with the virus, a humoral immune response produces the antibody. In general, IgM antibodies rise a few days after a viral infection and can be detected after a week of incubation, and IgG antibodies are known to appear in the middle and late stages of infection. There is a continuous increase known in the antibody titer and remains in the bloodstream for a long time. The supplementary antibody test can make up for the missed diagnosis of RT-PCR. However, it still cannot diagnose all infected patients.<sup>6</sup> The IgM and IgG testing, in combination, are known to be important to improve the clinical sensitivity of early COVID-19 diagnosis. Certainly, the detection sensitivity was confirmed to be higher in the IgG-IgM combined antibody test than in the individual IgG or IgM antibody test.<sup>9</sup> In our study, we found that the positive ratio of total IgM/IgG antibody detection in severe cases was 75.9% and in normal cases was 5.7%. Wang et al.,<sup>6</sup> stated that the detection of IgM and IgG antibodies could only achieve a sensitivity of 70% at 4 up to 6 days after admission for COVID-19 patients. Vidal-Anzardo et al.,<sup>5</sup> stated that the rapid serological tests were useful as a complementary test to PCR, especially during the second and third week of illness as well.

Since COVID-19 disease symptoms are not specific enough to diagnose, NAT (nucleic acid tests) to directly detect the targeted pathogen are applied as standard. However, high false negativity rates have been reported.<sup>10,11</sup> In addition, problems are encountered such as RT-PCR molecular tests take a long time, difficulties encountered in the application, the application only in reference laboratories, being expensive, requiring trained personnel, only detecting acute infection. CT was used in patients with COVID-19 suspected clinical signs to confirm RT-PCR tests. When only CT is used in the diagnosis of COVID-19, it can be confused with pneumonia caused by another viral infection agent. Serological tests - ELISA (Enzyme-dependent Immune Assay), in addition to NATs, are essential for monitoring surveillance studies. This is important in identifying both the sick and healed individuals and knowing the immune status of the community. However, serological tests are not suitable for the diagnosis of acute disease. When seropositivity occurs, SARS-CoV-2 antibodies and secreted antibodies for other Coronavirus infections may cross-react. Therefore, it has no value for early diagnosis. In clinical cases, it can only be valuable for determining immunity or useful in answering epidemiological questions.<sup>12</sup> Serological tests are not useful in diagnosing acute cases. Because, the first week of the disease, IgM and IgG antibody response is insufficient and only detected after about 6-15 days from the onset of the disease reaches the acceptable level.<sup>13</sup>

[Table 2](#) shows the clinical features of SARS-CoV-2 patients. The most symptoms observed in mild group were cough (65.6%), respiratory distress (26.2%), malaise (45.9%), sore throat (18%) and headache (8.2%), in moderate group were cough (81.3%), malaise (32.1%), respiratory distress (30.4%), fever (25.0%), sore throat (14.3%) and headache (8.9%), in severe group were respiratory distress (48.1%), malaise (40.7%), fever (31.5%), headache and nausea/vomiting (13%), myalgia (25.9%), stomach pain (7.4%) and loss of taste/smell (3.7%) and in normal group were cough (61.3%), malaise (29.2%), respiratory distress (28.3%), fever (17.9%) and headache and myalgia (10.4%).

It is known that the aged population (age above 65 years), and persons who have decreased immunity or chronic diseases, such as cardiovascular diseases, diabetes mellitus, hypertension, and lung disease, are at a higher risk for developing severe COVID-19 if they are infected with SARS-CoV-2. Therefore, to prevent the infection, they must take special precautions.<sup>14</sup> In our study, cardiovascular diseases were detected 4.9%(n=3) of mild, 5.4%(n=6) moderate, 7.4%(n=4) of severe and 0.9%(n=1) normal cases, pulmonary diseases were detected 3.6%(n=4) of moderate, 11.1%(n=6) of severe and 1.9%(n=2) of normal cases, diabetes mellitus were detected 6.6% (n=4) of mild, 8.9%(n=10) of moderate, 14.8%(n=8) of severe and 4.7%(n=5)

of normal cases, cancer were detected 0.9%(n=1) of moderate, 3.7%(n=2) of severe and 0.9% of normal cases (n=1) and hypertension were detected 9.8% (n=6) of mild, 7.1% (n=8) of moderate, 13%(n=7) of severe and 2.8(n=3) of normal cases. In a recent study, Özüdoğru et al.,<sup>15</sup> reported that diabetes mellitus, cardiovascular diseases, pulmonary diseases and hypertension detected 15.4%, 5.8%, 13.5%, 17.3% of 52 SARS-CoV-2 patients, respectively. In another recent study, Yung et al.,<sup>16</sup> found that diabetes mellitus, cardiovascular diseases, pulmonary diseases and hypertension detected 22%, 5%, 8%, 17% of 52 SARS-CoV-2 patients, respectively. In recent studies, Guan et al.,<sup>17</sup> Zhang et al.,<sup>18</sup> Zhou et al.,<sup>19</sup> and Yen et al.,<sup>20</sup> also detected some chronic diseases in COVID-19 patients. In our study, chronic diseases were detected more in severe cases, suggesting that persons who have decreased immunity or chronic diseases, such as cardiovascular diseases, diabetes mellitus, hypertension, and lung disease, are at a higher risk for developing severe COVID-19 if they are infected with SARS-CoV-2.

#### 4. CONCLUSION

In conclusion, according to the results that we obtained from this study, we can suggest a higher detection sensibility in RT-PCR than in IgM/IgG antibody test for mild, moderate and normal group, while the detection sensibility of IgM/IgG antibody increases in a severe group with diffuse bilateral involvement according to CT. The use of IgM/IgG antibody tests may contribute significantly to improve the accuracy of the clinical diagnosis, especially in hospitalized patients with negative molecular test results and in patients who have just undergone RT-PCR. Instead of using the tests as an alternative to each other, using them as complementary will be indispensable for definitive diagnosis. If it is necessary to diagnose with the IgG-IgM combined serological and/or molecular tests specifically for the patient's condition and to evaluate the CT findings together with the biomarkers to be detected from the patient's blood will help us get through this pandemic process best. The immediate priority for the diagnosis of COVID-19 is the development of nucleic acid and protein tests and their integration into multiplex panels over time. To improve surveillance efforts, to support patient-healing and vaccination studies, it is best to use test methods appropriate for the period of the disease and to verify with clinical findings.

#### DISCLOSURE STATEMENT

The authors reported no potential conflict of interest.

#### FUNDING INFORMATION

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## SHORT BIOGRAPHY



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Table 1. Comparison of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and total IgM-IgG antibody test for the diagnosis of SARS-CoV-2 infection

	Results				
	Mild (n=60)	Moderate (n=111)	Severe (n=53)	Normal (n=105)	Total (n=329)
IgM/IgG (+)	39.3% (n=24)	54.5%(n=61)	75.9%(n=41)	5.7%(n=6)	40%(n=132)
SARS-CoV-2 RNA (RT-PCR) (+)	80.3% (n=49)	85.7%(n=96)	75.9%(n=41)	95.3%(n=101)	87%(n=287)
Age-range	17-77 (SD:15.577)	19-99(SD:16.001)	20-92 (SD:15.452)	17-84(SD:14.251)	17-99 (SD:16.831)
Mean (age)	38.4(SE:2.011)	45.05 (SE:1.519)	55.5(SE:2.122)	34.8(SE:1.391)	42.25 (SE:0.928)
Median (age)	34	43	55	32	40
Male	52.5% (n=32)	49.1%(n=55)	53.7%(n=29)	49.1%(n=52)	50.15%(165)
Female	45.9% (n=28)	50%(n=56)	44.4%(n=24)	50%(n=53)	49.85%(164)

Table 2. Clinical characteristics of patients with COVID-19 pneumonia

Symptoms	Mild (n=60)	Moderate (n=111)	Severe (n=53)	Normal (n=105)	Total (n=329)
Fever	18%(n=11)	25.0% (n=28)	31.5%(n=17)	17.9%(n=19)	22.7% (n=75)
Cough	65.6% (n=40)	81.3% (n=91)	81.5%(n=4)	61.3%%(n=65)	72.7% (n=240)
Respiratory distress	26.2% (n=16)	30.4% (n=34)	48.1% (n=26)	28.3%(n=30)	32.1% (n=106)
Sore throat	18% (n=11)	14.3%(n=16)	3.7%(n=2)	17.9%(n=19)	14.5%(n=48)
Malaise	45.9% (n=28)	32.1% (n=36)	40.7% (n=22)	29.2% (n=31)	35.5%(n=117)
Headache	8.2%(n=5)	8.9%(n=10)	13.0%(n=7)	10.4%(n=11)	10%(n=33)
Nausea/vomiting	6.6%(n=4)	4.5%(n=5)	13.0%(n=7)	1.9%(n=2)	5.5% (n=18)
Diarrhea	6.6 (n=4)	2.7%(n=3)	7.4%(n=4)	2.8%(n=3)	4.2%(n=14)
Myalgia	14.8% (n=9)	14.3% (n=16)	25.9% (n=14)	10.4% (n=11)	15.2%(n=50)
Stomach pain	3.3%(n=2)	2.7%(n=3)	7.4%(n=4)	3.8%(n=4)	3.9%(n=13)
Loss of taste/smell	1.6%(n=1)	0.9%(n=1)	3.7%(n=2)	1.9%(n=2)	1.8%(n=6)
<b>Comorbidities</b>					
Cardiovascular disease	4.9%(n=3)	5.4%(n=6)	7.4%(n=4)	0.9%(n=1)	4.2%(n=14)
pulmonary disease	0	3.6%(n=4)	11.1%(n=6)	1.9%(n=2)	3.6%(n=12)
Diabetes	6.6%(n=4)	8.9%(n=10)	14.8%(n=8)	4.7%(n=5)	8.2%(n=27)
Hypertension	9.8% (n=6)	7.1%(n=8)	13%(n=7)	2.8(n=3)	7.3%(n=24)
Cancer	0	0.9%(n=1)	3.7%(n=2)	0.9%(n=1)	1.2%(n=4)

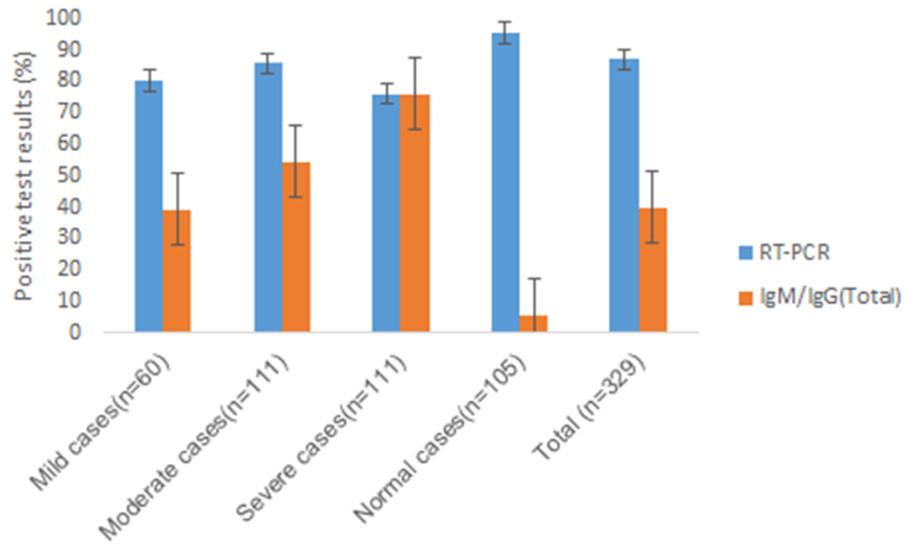


Figure 1. Positive percentages of RT-PCR and IgM-IgG tests by groups