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Original article

Prolonged viral shedding and antibody persistence in patients with COVID-19

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ABSTRACT

SARS-CoV-2 as a new global threat has affected global population for one year. Despite the great effort to eradicate this infection, there are still some challenges including different viral presentation, temporal immunity in infected individuals and variable data of viral shedding. We studied 255 COVID-19 suspected individuals to assess the viral shedding duration and also the antibody development against SARS-CoV-2 among the cases. Real Time RT-PCR assay was applied to determine the virus presence and SARS-CoV-2 antibodies were evaluated using SARS-CoV-2 IgM and IgG kits. 113 patients were confirmed for COVID-19 infection. The patients were followed until negative PCR achieved. The median viral shedding among studied population was obtained 34.16 (±17.65) days which was not significantly associated with age, sex and underlying diseases. Shiver and body pain were found in prolonged form of the infection and also patients who had gastrointestinal problems experienced longer viral shedding. Moreover, IgG was present in 84% of patients after 150 days. According to this data, the median viral shedding prolongation was 34.16 days which indicates that 14 days isolation might not be enough for population. In addition, IgG profiling indicated that it is persistent in a majority of patients for nearly 6 months which has brought some hopes in vaccine efficacy and application.

The emergence of COVID-19 has become a global health threat worldwide since the pandemic started in December, 2019. Awareness of SARS-CoV-2 transmission dynamics could have significant implications for hospitalization and infection prevention to control the disease. According to the variable data which is emerging rapidly, inpatient and outpatient isolation span has been controversial. Therefore, a clear vision of the viable viral shedding duration is critically important to provide a unique guidance for transmission-based isolation precautions [1].

The viral detection by PCR, is dependent on the time post illness initiation. In the first two weeks of the infection phase, the virus could be detected mostly in sputum followed by nasal swabs, while throat swabs were assessed unreliable eight days after symptom onset [2,3]. A high viral shedding rate has been found during the first week of symptoms with a peak on the fourth day [4]. Viral

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shedding prolongation is among the current COVID-19 challenges which has been uncharacterized after symptoms resolution [5].

Some studies have demonstrated that prolonged viral shedding is correlated with severe presentation of the virus. Zheng et al. in a retrospective cohort study on 96 patients observed prolonged viral shedding in severe cases in comparison with mild cases [6,7]. In a study on MERS-CoV, diabetes was found to be correlated with prolonged detection of MERS-CoV RNA [8]. A case report also showed that a COVID-19 infected case had viral shedding for 46 days who was suffering from chronic hepatitis B infection and diabetes mellitus [9]. High fever at the time of admission also resulted in longer SARS-CoV-2 shedding. In addition to social distancing and quarantining of confirmed cases and contacts, viral shedding duration determination will help reducing viral transmission. According to unclear features of the new virus, determination of the viral shedding in different populations is essential to determine an effective standard protocol in recovered patients' discharge [10–12].

Furthermore, antibody measurement is a crucial key which provides essential data on infection tracking. Population based serology could make a clear vision of the virus spreading pattern and total attack rate along with the prevalence of serological conversion [13,14]. Moreover, individual serology testing may reveal unsuspected previous exposure which indirectly could indicate presence of the virus among a population. In addition, the combination testing of PCR and antibody measurement might significantly enhance the opportunity to detect present and past infection [15,16]. Therefore, it is important to understand the key features of serology testing including the pattern of seroconversion over time, the first day of detectable antibodies, differences between antibodies wax and wane and duration of antibody response among COVID-19 infection. Determination of immune and non-immune SARS-CoV-2 individuals among a community based on serological test will help us to estimate epidemiological variables including case-fatality and attack rate and also to identify subjects who mounted a strong virus specific antibody response which can be then detected and applied to treat patients via plasma therapy [17,18].

Serological testing may also identify patients with past infection without PCR positivity and has been used in surveillance to identify previous SARS-CoV-2 infection and provide the infectious link between known cases. This kind of test may also provide better estimate of past SARS-CoV-2 infection among the community [5].

Despite seroconversion, viral shedding has been shown to persist and virus has also been cultured after SARS-CoV-2 antibodies detection [19,20]. Nearly up to half of SARS-CoV-2 patients have been reported to develop an antibody response on the 7th day with the vast majority seroconverting 15 days after symptom onset [19,21].

In this study, we aimed at characterization of SARS-CoV-2 viral shedding and antibody assessment among Iranian COVID-19 infected subjects.

2. Materials and methods

In this study, 255 COVID-19 suspected individuals who referred to Pasteur Institute of Iran from March 2020 to September 2019 were enrolled. The SARS-CoV-2 was assessed by Viral RNA extraction using a QIAcube HT system with a QIAamp96 Virus QIAcube HT Kit, according to the manufacturer's instructions. Real Time Reverse-transcription PCR (Real Time RT-PCR) assay was performed using 2019-nCoV Nucleic Acid Diagnostic kit (Sansure biotech, Changsha, China), according to the manufacturer's protocol and using the designed primers (Table 1). Upon admission and SARS-CoV-2 specific antibody was evaluated using SARS-CoV-2 IgM Capture kit, based on Nucleocapsid and Spike antigens (cat no: PT-CoV-2-IgM Cap-96, Pishtazteb, Iran) and SARS-CoV-2 IgG kit, based on applying SARS-CoV-2 N antigen (cat no: PT-CoV-19 IgG-96, Pishtazteb, Iran) according to the provided protocol. Demographic data, clinical findings, therapeutic regimen, underlying diseases and viral presentation of the patients recorded in questionnaire. Viral clearance was confirmed by two sets of RT-PCR after 24 h for each positive individual.

All the procedures were in accordance with the ethical standards of the responsible committee on human experimentation of Pasteur Institute of Iran under IR.PII.REC.1399.009 ethical code and with the Helsinki Declaration.

The COVID-19 disease was divided to mild, moderate and severe forms as: Individuals who had any of the various signs and symptoms of COVID-19 (fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but not shortness of breath, dyspnea, or abnormal chest imaging were considered mild cases. Subjects who showed evidence of lower respiratory disease during clinical assessment or imaging and with saturation of oxygen (SpO2) $\geq$94% on room air at sea level were moderate. The patients with SpO2 <94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mmHg, respiratory frequency >30 breaths per minute, or lung infiltrates >50% were considered severe. In this study, the suspicious subjects were tested by PCR and the positive detected cases were investigated for viral presentation and other demographic data. The two groups of negative PCR and positive PCR cases were then compared in term of age, gender, and the related risk factors including underlying diseases. The infected individuals were also fully studied by the possible related risk factors to the viral shedding and antibody development and persistence.

Data are presented as mean ± SD or, when indicated, as an absolute number and percentage. The Chi-square, T-student, one-way ANOVA and Fisher tests were applied using SPSS 26 package program for statistical analysis. P value less than 0.05 was considered significant.

3. Results

3.1. Studied population

Totally 255 suspected subjects (133 males, 122 females) were evaluated. 238 cases were assessed by molecular tests from whom 113 patients were confirmed for the COVID-19 infection. The mean age was 40.65 (±13.99) and the O and A blood types were the most frequent respectively.

Table 1
The PCR primers and program applied in the study.

N gene	N1F primer	GACCCCAAAATCAGCGAAATG	
	N3R primer	TGTAGCACGATTGCAGCATTG	
S gene	Zabih OF	TCAGACAAATCGCTCCAGGG	
	Zabih OR	AGCAACTGAATTTTCTGCACCA	
	Temp.	Duration	Number of cycles
PCR program	50 °C	20 min	8
	95 °C	2 min	
	95 °C	15 s	
	65 °C	20 s	
	72 °C	1 min	
	95 °C	15 s	35
	58 °C	20 s	
	72 °C	1 min	
	72 °C	5 min	

Anosmia, loss of taste, fever and body pain were significantly higher in the positive PCR compared to the negative ones ($P < 0.05$). The other outcome of the analytic data was that there is an association between the age and the infection and older people were more susceptible to acquire the disease ($p < 0.001$). Moreover, infected population of males were twofold females. There was no correlation between the underlying disease and COVID-19 acquiring in the confirmed cases and non-infected subjects (Table 2).

3.2. Viral shedding assessment

The patients were followed until the disease resolution achieved by PCR test (Table 3). The earliest viral clearance was assessed 5 days whereas 91 days as the longest period after symptoms onset. The median viral shedding was obtained 34.16 (±17.65) days which was not significantly different between the men and women. Moreover, the studied population were divided to two groups based on the age, >50 years and <50 years. The mean duration of viral shedding was 36.9 (±24.15) and 34.84 (±15.59) days in the groups, respectively with no significant differences between them.

Among the investigated population, viral clearance was followed up weekly which showed 43.3% achieved it within 28 days whereas just 6.7% achieved negative PCR in14 days.

There was a significant association between some of the disease presentations and prolongation of the virus shedding. Shiver and body pain were found in prolonged form of the infection and also patients who had gastrointestinal problems (including abdominal pain, diarrhea, anorexia and vomiting) experienced prolonged viral shedding, too (Table 4).

What is more, 9 patients (3.5%) used Chloroquine and also 44 subjects got Hydroxychloroquine. Nevertheless, there was no association between the viral shedding and Chloroquine/Hydroxychloroquine usage (Table 4).

3.3. SARS-CoV-2 Antibody evaluation

The serological data of IgM and IgG were available for 77 and 78 patients, respectively. 35.1% developed IgM with the median days of 28.5 from symptoms onset. Comparison among different groups of patients showed that IgM was produced lately in those who experienced headache and taste lost (Table 4).

From the IgM positive patients, 12 individuals were followed until IgM decay. The data showed that IgM median persistence was 107.25 days with the maximum time of 150 days. The patients who were treated by chloroquine achieved more persistent IgM compared to the others ($p < 0.04$). What is more, the IgM faint was observed in males two times sooner than females. The individuals with O blood type were found to lose IgM sooner than the other groups ($p = 0.01$). There was no association between the gender and blood types with antibody detection.

Furthermore, 25 patients from those who were IgG positive agreed to participate in serial testing antibody within at least 5 months which the obtained data showed that 84% were IgG positive whereas 1 individual lost IgG after 5 months and the other case lost it after 6 months. Moreover, IgG was not detected in one patient and in one case it was only present for 1 month.

In addition, there was no association between the disease severity and IgG raise time. Finally, the subjects who did not suffer from earache reached the IgG development sooner than the rest.

4. Discussion

Determination of SARS-CoV-2 shedding and also the correlated antibody persistence are two crucial matters in this era. The clear vision of how long the immune responses protect the host from re-infection and how well it works has not achieved yet. Indeed, understanding of the virus different features and presentation in individuals are still surprising. Therefore, more investigated individuals and the virus characterization will reflect on quarantine guidelines development and viral transmission prevention [22].

In this study we investigated the median time of viral shedding in COVID-19 infected subjects and also the antibody response in the sub groups. The median viral shedding among the individuals was 34.16 (±17.65) days which was associated with some viral presentation including shiver, body pain and gastrointestinal problems. Moreover, viral clearance following up indicated that the majority of the subjects had negative PCR test after 36 days. Therefore, the importance of PCR test in the patient's resolution confirmation seems essential as it varies between individuals.

According to some classified data, SARS-CoV-2 viral shedding has been continued for 63 days after symptom initiation and SARS-CoV-2 RNA was detectable in infected cases 1–3 days earlier than symptom starts. Moreover, the disease severity had no effect on viral RNA detection even in upper or lower respiratory tract although a correlation was observed between longer viral RNA detection and severe form of the illness [23].

In a study by Mancuso et al., 1162 subjects testing positive to RT-PCR were followed for 30 days. 60.6% of the subjects achieved viral clearance with a median time of 36 days from the symptoms onset. There was an association between the age and the viral clearance which increased slightly in older patients (>80 years) [24].

In a study by Qi et al. on 147 patients with COVID-19 the median duration of viral shedding was assessed as 17 days. The high temperature at the time of admission and hospital stay length were found as correlated risk factors to prolonged viral shedding [10].

In a study by Fu Y et al., on 410 COVID-19 confirmed cases who were discharged, 96% of the subjects were RT-PCR negative within 30 days after symptom onset. The associated risk factor with viral shedding was found coronary heart disease (CHD) which led to prolonged viral RNA shedding. Moreover, patients with lower albumin levels experienced prolonged viral RNA shedding [25].

According to our data, the median viral shedding was obtained 34.16 (±17.65) days which is in accordance with Mancuso et al. study. Unlike the Mancuso et al. findings, our results indicated that there was no significant correlation between the age and median of viral shedding. However, some symptoms including shiver, body pain and gastrointestinal problems were associated with prolonged viral shedding in our study. Moreover, in contrast Fu et al. report, we found no correlation between underlying diseases and the median duration of SARS-CoV-2 shedding.

Some data have indicated that underlying disease might led to a high risk of COVID-19 infection or the more severe form of it. In a study conducted on 18,940 study Korean participants, the BMI level and its association to acquire COVID-19 was aimed. It was shown that, the overweight individuals were in a higher rate of SARS-CoV-2 risk comparing to the normal subjects [26]. Moreover, patients with chronic kidney and lung disease are at an increased risk of severe COVID-19 which could stem from the host microbiome, excessive mucous production and alterations in the systemic/local immune response [27,28]. Nevertheless, in this study, we did not find any significant association between the underlying diseases and COVID-19 acquisition which might be correlated with the low number of participants comparing with the studies above.

Antibody assessments a key point provides essential data on infection tracking. Different population based serology can provide a clear vision of serological persistence and also its conversion. We followed the patients with IgG positive in serial testing antibody within 6 months. The data showed that IgG was positive in 84% whereas 2 individuals lost IgG in 5 and 6 months. This rate of IgG

Table 2
Demographic characteristics of the COVID-19 patients.

	Total	Negative	Positive	OR (95% CI)	P value
Demographic characteristics					
Age	40.61 ± 14.01	37.34 ± 13.36	44.57 ± 13.94		<0.001
Gender (Male)	128 (52.7.0)	60 (46.2)	68 (60.2)	1.76 (1.06–2.94)	**0.03**
Blood group (n = 182)					
A	63 (34.6)	31 (31.3)	32 (38.6)	1	0.06
O	67 (36.8)	40 (40.4)	27 (32.5)	0.65 (0.33–1.31)	
B	31 (17.0)	21 (21.2)	10 (12.0)	0.46 (0.19–1.14)	
AB	21 (11.5)	7 (7.1)	14 (16.9)	1.94 (0.69–5.44)	
Rh+	163 (90.1)	87 (88.8)	76 (91.6)	1.37 (0.51–3.72)	0.53
contact with cases	163 (70.6)	94 (75.2)	69 (65.1)	0.62 (0.35–1.09)	0.09
Severity					
Mild	70 (44.3)	30 (63.8)	40 (36.0)	1	**0.004**
Moderate	84 (53.2)	17 (36.2)	67 (60.4)	2.96 (1.45–6.03)	
Severe	4 (2.5)	0 (0.0)	4 (3.6)	–	
Symptoms					
fever	69 (28.4)	24 (18.5)	45 (39.8)	2.92 (1.63–5.23)	<**0.001**
Chills	77 (31.7)	27 (20.8)	50 (44.2)	3.03 (1.72–5.32)	<**0.001**
Sweating	88 (36.4)	39 (25.4)	55 (49.1)	2.84 (1.65–4.87)	<**0.001**
Cough	88 (36.4)	39 (30.0)	49 (43.8)	1.82 (1.07–3.08)	**0.03**
Sputum	62 (25.5)	25 (19.2)	37 (32.7)	2.05 (1.14–3.68)	**0.02**
Shortness of breath	64 (26.3)	27 (20.8)	37 (32.7)	1.86 (1.04–3.31)	**0.04**
body pain	110 (45.3)	42 (32.3)	68 (60.2)	3.17 (1.87–5.36)	<**0.001**
Chest pain	62 (25.5)	30 (23.1)	32 (28.3)	1.32 (0.74–2.35)	0.35
Lethargy and fatigue	115 (47.3)	46 (35.4)	69 (61.1)	2.87 (1.70–4.83)	<**0.001**
runny nose	52 (21.4)	20 (15.4)	32 (28.3)	2.17 (1.20–4.07)	**0.01**
Sore throat	72 (29.6)	41 (31.5)	31 (27.4)	0.82 (0.47–1.43)	0.49
Diarrhea	42 (17.3)	14 (10.8)	28 (24.8)	2.73 (1.36–5.50)	**0.004**
Nausea	37 (15.2)	13 (10.0)	24 (21.2)	2.43 (1.17–5.03)	**0.02**
Vomiting	12 (4.9)	4 (3.1)	8 (7.1)	2.40 (0.70–8.19)	0.15
Stomach ache	33 (13.6)	12 (9.2)	21 (18.6)	2.25 (1.05–4.80)	**0.03**
Anorexia	59 (24.8)	19 (14.6)	40 (35.4)	3.20 (1.72–5.96)	<**0.001**
Headache	101 (41.6)	42 (32.3)	56 (52.2)	2.28 (1.36–3.85)	**0.002**
Anosmia	71 (29.2)	19 (14.6)	52 (46.0)	4.98 (2.70–7.18)	<**0.001**
Loss of taste	45 (18.5)	10 (7.7)	35 (31.0)	5.39 (2.52–11.50)	<**0.001**
Dry throat	67 (27.6)	30 (23.1)	37 (32.7)	1.62 (0.92–2.86)	**0.09**
Eye pain	20 (8.2)	5 (3.8)	15 (13.3)	3.83 (1.34–10.89)	**0.01**
Earache	36 (14.9)	18 (13.8)	18 (16.1)	1.19 (0.59–2.42)	0.63
Hypotension	21 (8.7)	10 (7.8)	11 (9.7)	1.28 (0.52–3.15)	0.59
Agitation	40 (16.8)	12 (9.2)	28 (24.8)	3.24 (1.56–6.73)	**0.01**
Itch	23 (9.5)	12 (9.2)	11 (9.7)	1.06 (0.50–2.51)	0.89
Depression	21 (8.6)	8 (6.2)	13 (11.5)	1.99 (0.79–4.97)	0.14
Underling disease					
All types	47 (20.6)	22 (17.6)	25 (22.3)	1.35 (0.71–2.55)	0.36
Heart disease	15 (6.2)	6 (4.6)	9 (8.0)	1.79 (0.62–5.19)	0.24
Lung disease	17 (7.0)	9 (6.9)	8 (7.1)	1.03 (0.39–2.78)	0.95
Immunodeficiency disorders	5 (2.1)	3 (2.3)	2 (1.8)	0.79 (0.13–4.67)	0.99
Kidney disease	5 (2.1)	1 (0.8)	4 (3.5)	4.73 (0.52–42.99)	0.13
Diabetes	9 (3.7)	5 (3.8)	4 (3.75)	0.92 (0.24–3.50)	0.99
Hypertension	19 (7.8)	7 (5.4)	12 (10.6)	2.09 (0.79–5.50)	0.13

The P value < 0.05 are show in bold.

Table 3
Duration to achieve negative PCR test.

		Valid Percent	Cumulative Percent
Duration	<7 days	1.6	1.7
	8–14 days	4.9	6.7
	15–21 days	21.3	26.7
	22–28 days	16.4	43.3
	29–35 days	9.8	53.3
	More than 36 days	45.9	100.0
	Total	100.0	

development and persistent is greatly hopeful in clinical implication, vaccine development and re-infection concerns. In addition, there was no association between the disease severity and IgG raise time.

Antibody responses to the spike (S) protein of SARS-CoV-2 were evaluated in 343 North American patients that persisted up to 122 days after symptom onset. Moreover, the median time to seroconversion was about 12 days. IgA and IgM antibodies against RBD were short-lived. In contrary, anti-RBD IgG responses fainted slowly through 90 days. They concluded that IgG antibodies to SARS-CoV-2 RBD were strongly associated with anti-S neutralizing antibody titers [29]. Among the following-up in our study, 84% showed long-lived IgG among 6 months in parallel with the results of the American study results.

Table 4
Viral shedding and antibody development association with patients 'presentations.

	Sub group	The period between the symptoms onset to negative PCR		The period between the symptoms onset to IgM detection		IgM persistence		The period between the symptoms onset to IgG detection	
		Mean ± SD	P Value	Mean ± SD	P Value	Mean ± SD	P Value	Mean ± SD	P Value
Gender	Female	32.53 ± 17.95	0.47	41.00 ± 22.78	0.13	117.83 ± 45.08	0.53	113.84 ± 63.94	0.17
	Male	36.18 ± 17.61		28.11 ± 7.99		96.67 ± 65.08		87.86 ± 64.88	
Blood group	A	39.91 ± 23.38	0.57	36.80 ± 14.18	0.92	116.00 ± 62.55	0.61	101.47 ± 59.64	0.53
	O	33.79 ± 13.68		31.17 ± 10.28		103.00 ± 78.72±78.72		103.92 ± 63.20	
	B	40.83 ± 15.55		41.00 ± 42.44		82.50 ± 45.00		106.00 ± 85.59	
	AB	30.57 ± 10.74		33.00 ± 10.44		150.00 ± 0.00		109.20 ± 62.58	
Rh	Rh-	35.60 ± 26.23	0.87	24.00	–	–	–	58.00 ± 62.74	0.09
	Rh+	37.09 ± 17.89		35.56 ± 18.71		107.25 ± 54.51		113.03 ± 60.61	
Contact with suspicious cases	No	40.48 ± 19.52	0.11	26.89 ± 10.39	0.08	112.67 ± 61.28	0.75	110.17 ± 67.83	0.36
	Yes	32.00 ± 16.66		42.50 ± 22.31		52.07 ± 21.26		92.04 ± 63.47	
Chloroquine	No	34.19 ± 17.22	0.90	35.00 ± 19.35	–	75.43 ± 50.44	0.04	92.16 ± 65.54	0.97
	Yes	35.17 ± 17.69		29.00 ± 00		153.00 ± 14.00		90.83 ± 69.28	
Hydroxy chloroquine	No	32.21 ± 15.85	0.19	35.45 ± 19.45	0.78	102.43 ± 61.34	0.77	88.52 ± 64.91	0.66
	Yes	38.35 ± 19.11		32.25 ± 19.05		90.00 ± 51.96		97.81 ± 67.43	
Severity of disease	Mild	32.04 ± 16.92	0.40	–		–		65.68 ± 16.96	0.75
	Moderate	37.61 ± 17.56		–		–		65.03 ± 12.08	
	Severe	43.50 ± 31.82		–		–		79.90 ± 56.50	
Fever	No	32.18 ± 17.13	0.14	34.36 ± 20.61	0.96	103.86 ± 62.01	0.81	103.83 ± 66.42	0.46
	Yes	38.93 ± 17.91		34.86 ± 13.93		112.00 ± 48.52		89.47 ± 63.82	
Chills	No	30.35 ± 17.34	**0.03**	28.80 ± 13.33	0.13	83.60 ± 62.95	0.22	88.74 ± 66.54	0.26
	Yes	40.13 ± 16.84		41.75 ± 20.93		124.14 ± 44.79		110.24 ± 62.71	
Sweating	No	30.93 ± 16.22	**0.08**	28.25 ± 8.50	0.19	89.50 ± 71.07	0.45	96.64 ± 67.38	0.88
	Yes	39.00 ± 18.50		39.60 ± 21.94		116.13 ± 47.27		99.42 ± 64.43	
Cough	No	33.84 ± 16.90	0.47	37.67 ± 20.58	0.31	110.75 ± 57.21	0.77	104.24 ± 65.75	0.24
	Yes	37.26 ± 19.37		28.33 ± 8.96		100.25 ± 56.20		80.93 ± 62.45	
Sputum	No	31.28 ± 16.63	0.63	35.86 ± 19.91	0.58	103.11 ± 57.60	0.67	97.18 ± 64.44	0.87
	Yes	36.73 ± 19.65		30.00 ± 7.12		119.67 ± 52.54		100.50 ± 69.10	
Shortness of breath	No	35.10 ± 16.00	0.93	33.50 ± 18.61	0.65	99.10 ± 56.23	0.27	103.42 ± 64.36	0.34
	Yes	35.32 ± 21.34		38.25 ± 16.70		148.00 ± 15.56		82.33 ± 67.57	
Body pain	No	29.27 ± 14.36	**0.02**	26.43 ± 10.05	0.13	74.33 ± 69.62	0.25	99.00 ± 65.75	0.94
	Yes	39.54 ± 18.76		39.73 ± 20.12		118.22 ± 48.75		97.54 ± 65.84	
Chest pain	No	33.00 ± 16.86	0.16	29.00 ± 8.67	0.10	120.88 ± 57.85	0.24	97.80 ± 65.99	0.95
	Yes	39.95 ± 18.89		43.29 ± 25.12		80.00 ± 20.0		99.08 ± 65.28	
Lethargy and fatigue	No	30.23 ± 15.71	0.06	28.00 ± 9.14	0.22	104.33 ± 79.10	0.92	103.94 ± 67.48	0.64
	Yes	38.83 ± 18.34		38.73 ± 21.02		108.22 ± 50.17		94.67 ± 64.55	
runny nose	No	34.26 ± 16.71	0.47	32.40 ± 11.66	0.26	100.00 ± 61.23	0.54	94.65 ± 65.22	0.41
	Yes	38.21 ± 20.92		45.33 ± 39.31		121.75 ± 41.54		115.63 ± 65.89	
Sore throat	No	33.63 ± 15.33	0.24	33.87 ± 18.63	0.67	106.40 ± 55.11	0.94	104.11 ± 64.98	0.30
	Yes	39.89 ± 23.44		38.00 ± 16.73		111.50		82.08 ± 65.34	
Diarrhea	No	31.60 ± 14.38	**0.01**	34.80 ± 19.44	0.90	93.11 ± 56.24	0.12	96.66 ± 65.79	0.80
	Yes	45.19 ± 22.25		33.33 ± 7.57		149.67 ± 9.50		102.15 ± 65.97	
Nausea	No	32.17 ± 15.37	**0.01**	30.38 ± 12.31	0.11	108.38 ± 59.22	0.93	94.69 ± 63.28	0.53
	Yes	46.23 ± 21.56		45.40 ± 26.36		105.00 ± 51.96		72.20 ± 20.84	
Vomiting	No	33.82 ± 16.10	**0.046**	–	–	–	–	99.51 ± 65.40	0.58
	Yes	50.20 ± 28.35		–		–		77.67 ± 69.62	
Stomachache	No	32.04 ± 14.27	0.004	35.13 ± 19.11	0.77	103.36 ± 55.40		101.40 ± 64.65	0.45
	Yes	47.92 ± 24.29		31.67 ± 11.59		150.00		81.88 ± 69.38	
Anorexia	No	30.29 ± 14.55	**0.001**	28.73 ± 8.52	0.08	103.40 ± 68.95	0.85	96.96 ± 69.39	0.86
	Yes	45.95 ± 19.46		43.71 ± 24.88		110.00 ± 47.50		99.80 ± 60.32	
Headache	No	33.19 ± 17.38	0.36	26.50 ± 8.33	0.03	85.40 ± 65.40	0.26	103.41 ± 67.391	0.61
	Yes	37.34 ± 18.00		44.63 ± 21.80		122.86 ± 43.74		93.69 ± 64.10	
Anosmia	No	32.53 ± 17.35	0.14	29.27 ± 8.63	0.12	81.00 ± 59.70	0.17	89.76 ± 63.0	0.28
	Yes	39.52 ± 17.67		42.86 ± 25.42		126.00 ± 45.68		110.95 ± 67.83	
Loss of taste	No	32.17 ± 15.57	**0.02**	31.38 ± 11.98	0.03	114.11 ± 57.79	0.48	95.78 ± 64.14	0.65
	Yes	44.33 ± 20.91		60.00 ± 42.43		88,367 ± 46.188		106.09 ± 70.85	
Dry throat	No	34.07 ± 16.17	0.48	31.54 ± 12.95	0.26	105.71 ± 63.79	0.91	89.63 ± 65.33	0.14
	Yes	38.58 ± 20.84		42.40 ± 27.25		109.40 ± 45.35		121.08 ± 61.06	
Eye pain	No	35.27 ± 18.27	0.88	–	–	–	–	104.40 ± 62.92	0.08
	Yes	34.00 ± 9.06			–	–		54.33 ± 68.89	
Earache	No	33.53 ± 16.13	0.06	34.00 ± 19.31	0.84	–		99.10 ± 67.04	0.82
	Yes	46.00 ± 24.19		36.00 ± 15.17		–		93.38 ± 58.24	
Hypotension	No	35.40 ± 18.08	0.79	32.40 ± 11.95	0.26	–		102.65 ± 65.33	0.16
	Yes	33.63 ± 15.51		45.33 ± 38.70		–		59.40 ± 53.77	
Agitation	No	32.28 ± 14.39	**0.01**	–	–	–	–	99.85 ± 66.13	0.69
	Yes	48.27 ± 24.98		–		–	–	89.63 ± 63.22	
Itching	No	34.43 ± 16.38	0.47	31.75 ± 11.83	0.06	116.70 ± 55.10	0.19	94.74 ± 66.14	0.32
	Yes	38.90 ± 23.86		46.67 ± 33.00		60.00 ± 0.00		117.86 ± 59.30	
Depression	No	34.36 ± 16.63	0.24	34.88 ± 18.61	0.84			98.27 ± 64.53	0.97
	Yes	44.20 ± 27.58		32.00 ± 14.14				98.75 ± 82.01	
Underlying Diseases	No	33.56 ± 13.78	0.18	32.46 ± 12.24	0.44	114.11 ± 57.95	0.46	99.43 ± 67.73	0.81
	Yes	41.08 ± 27.69		40.00 ± 29.18		85.67 ± 44.46		93.82 ± 58.14	

The P value < 0.05 are show in bold.

Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigen was studied by Baweleta Isho et al. According to the findings, anti-SARS-CoV-2 antibody responses were readily detected in serum and saliva, with peak IgG levels attained by 16–30 days after infection initiation. Longitudinal analysis showed that IgA and IgM antibodies quickly decayed whereas IgG antibodies persisted relatively stable up to 105 days in both biofluids. This study confirmed that IgG antibodies in serum and saliva are maintained in the majority of COVID-19 patients for at least 90 days [30].

In the other study by Figueiredo-Camposet al, antibody levels were assessed in hospitalized patients and volunteers who had recovered from SARS-CoV-2. The data showed that 90% of SARS-CoV-2–positive individuals developed detectable antibodies during 40–200 days post-infection, with higher levels in those presented more severe disease [31].

In this study we also found that IgG is present in 84% of patients after 180 days which is in agreement with the mentioned studies, although the neutralizing potency is not achieved.

As the studies are growing worldwide and the tests' specificity is more achieved, SARS-CoV-2 different presentations and host immune interaction are well understood. However, the different sampling patterns and variation in populations make it difficult to compare the related data.

Furthermore, IgG seroconversion and persistence should be investigated in a wider scope to come up with a unique evaluation scale. Although the IgG could persist for 6 months, it does not mean that the protection can also last for the same duration.

In conclusion, currently, 14 days isolation for the patients is recommended in regional surveillance protocols. Moreover, according to the last CDC recommendation, this duration could be even less (10 days) for mild form of the disease. It should be noted that viral detection upon swap sampling does not confirm the virus viability as it could be obtained from a dead particle. Contamination by PCR amplicons or reagents, sample cross-contamination and also cross-reactions with genetic material or other viruses could be responsible for false-positive results which could possibly maintained by double RT-PCR testing. According to the median viral shedding which is nearly 30 days, it seems that 14 days isolation might not be enough as some of the released patients could be still infectious. Antibody profiling data suggests that in most cases, IgG persists for nearly six months although it may last longer. Therefore, this data has the importance in vaccine application, too. The more studies are needed to evaluate antibody protection, establishment and also viral culture to determine its viability in order to design a more practical isolation guideline.

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Declaration of competing interest

Authors declare no potential conflict of interest that could negatively influence the study.

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References

[1] Fontana L, Villamagna AH, Sikka MK, McGregor JC. Understanding viral shedding of SARS-CoV-2: review of current literature. Infect Contr Hosp Epidemiol 2020;42:1–35.
[2] Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J, et al. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. medRxiv 2020:2020.2020.02.11.20021493.
[3] Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, et al. Diagnosing COVID-19: the disease and tools for detection. ACS Nano 2020;14(4):3822–35.
[4] He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 2020;26(5):672–5.
[5] Widders A, Broom A, Broom J. SARS-CoV-2: the viral shedding vs infectivity dilemma. Infect Dis Health 2020;25(3):210–5.
[6] Liu Y, Yan L-M, Wan L, Xiang T-X, Le A, Liu J-M, et al. Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis 2020;20(6):656–7.
[7] Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ 2020;369:m1443.
[8] Al-Abdely HM, Midgley CM, Alkhamis AM, Abedi GR, Lu X, Binder AM, et al. Middle East respiratory syndrome coronavirus infection dynamics and antibody responses among clinically diverse patients, Saudi Arabia. Emerg Infect Dis 2019;25(4):753–66.
[9] Liu Y, Chen X, Zou X, Luo H. A severe-type COVID-19 case with prolonged virus shedding. J Formos Med Assoc 2020;119:1555–7.
[10] Qi L, Yang Y, Jiang D, Tu C, Wan L, Chen X, et al. Factors associated with duration of viral shedding in adults with COVID-19 outside of Wuhan, China: a retrospective cohort study. Int J Infect Dis 2020;96:531–7.
[11] Mostafa Salehi-Vaziri TJ, Farahmand Behrokh, Fotouhi Fatemeh, Banifazl Mohammad, Hassan Pouriayevali Mohammad, Sadat Larijani Mona, et al. Clinical characteristics of SARS-CoV-2 by Re-infection Vs. Reactivation: a case series from Iran. Eur J Clin Microbiol Infect Dis 2021:1–7.
[12] Sofian M, Velayati AA, Banifazl M, Fotouhi F, Sadat Larijani M, Afzali N, et al. SARS-CoV-2, a virus with many faces: a series of cases with prolonged persistence of COVID-19 symptoms. Wien Med Wochenschr 2020;171:1–6.
[13] Burrell CJ, Howard CR, Murphy FA. Laboratory diagnosis of virus diseases. Fenner White's Med Virol 2017:135–54.
[14] Sadat SM, Aghadadeghi MR, Yousefi M, Khodaei A, Sadat Larijani M, Bahramali G. Bioinformatics analysis of SARS-CoV-2 to approach an effective vaccine candidate against COVID-19. Mol Biotechnol 2021;63:389–409.
[15] Tan AS, Nerurkar SN, Tan WCC, Goh D, Lai CPT, Poh Sheng Yeong J. The virological, immunological, and imaging approaches for COVID-19 diagnosis and research. SLAS Technol 2020;25:522–44.
[16] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 2020;71:2027–34.
[17] Jacofsky D, Jacofsky EM, Jacofsky M. Understanding antibody testing for COVID-19. J Arthroplasty 2020;35(7S):S74–81.
[18] Lee CY-P, Lin RTP, Renia L, Ng LFP. Serological approaches for COVID-19: epidemiologic perspective on surveillance and control. Front Immunol 2020;11(879).
[19] Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581(7809):465–9.
[20] Liu W-D, Chang S-Y, Wang J-T, Tsai M-J, Hung C-C, Hsu C-L, et al. Prolonged virus shedding even after seroconversion in a patient with COVID-19. J Infect 2020;81(2):318–56.
[21] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 2020;71:2027–34.
[22] Iwasaki A. What reinfections mean for COVID-19. Lancet Infect Dis 2021;21:3–5.
[23] Criteria for releasing COVID-19 patients from isolation [Internet]. World Health Organization (WHO). [cited 2020/6/26].
[24] Mancuso P, Venturelli F, Vicentini M, Perilli C, Larosa E, Bisaccia E, et al. Temporal profile and determinants of viral shedding and of viral clearance confirmation on nasopharyngeal swabs from SARS-CoV-2-positive subjects: a population-based prospective cohort study in Reggio Emilia, Italy. BMJ Open 2020;10(8):e040380.
[25] Fu Y, Han P, Zhu R, Bai T, Yi J, Zhao X, et al. Risk factors for viral RNA shedding in COVID-19 patients. Eur Respir J 2020;56:2001190.
[26] Jung C-Y, Park H, Kim DW, Lim H, Chang JH, Choi YJ, et al. Association between body mass index and risk of coronavirus disease 2019 (COVID-19): a nationwide case-control study in South Korea. Clin Infect Dis 2020.
[27] Henry BM, Lippi G. Chronic kidney disease is associated with severe coronavirus disease 2019 (COVID-19) infection. Int Urol Nephrol 2020;52(6):1193–4.
[28] Schultze A, Walker AJ, MacKenna B, Morton CE, Bhaskaran K, Brown JP, et al. Risk of COVID-19-related death among patients with chronic obstructive

pulmonary disease or asthma prescribed inhaled corticosteroids: an observational cohort study using the OpenSAFELY platform. Lancet Resp Med 2020;8(11):1106–20.

[29] Iyer AS, Jones FK, Nodoushani A, Kelly M, Becker M, Slater D, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. Sci Immunol 2020;5(52):eabe0367.

[30] Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol 2020;5(52):eabe5511.

[31] Figueiredo-Campos P, Blankenhaus B, Mota C, Gomes A, Serrano M, Ariotti S, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset. Eur J Immunol 2020;50:2025–40. n/a(n/a).