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Viral load and antibody levels in patients with COVID-19

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Abstract

Relations between viral load, antibody levels and COVID-19 severity are not well studied and results from such investigations are controversial. In this study, we investigated kinetics of viral load and antibody responses to SARS-CoV-2 in 20 patients with COVID-19 and analysed the association with disease severity. The patients were followed on weekly basis within the first month after the onset and then once per month for the next 4 months. Serum samples were tested for IgA, IgM, and IgG antibodies against SARS-CoV-2 using ELISA tests. SARS-CoV-2 viral load in nasopharyngeal swabs was measured by quantitative Realtime RT-PCR. For vast majority of the patients, the viral loads were at their highest levels at presentation and then declined gradually. Despite development of specific antibody response 7-11 days after the onset of COVID-19, SARS-CoV-2 RNA was still detected in nasopharyngeal swabs of most of the patients. There was no direct link between viral load and severity of COVID-19: some of mild and some of severe cases started with a high viral load. There was a relationship between the time from the onset of the disease and the viral load: the highest viral load was in the first days. In more severe cases, there was a tendency for slower reduction in viral load and longer detection of SARS-CoV-2 virus. Levels of the specific antibodies increased earlier and to higher levels and were present for longer time in patients with more severe manifestations of COVID-19 than in those with milder disease.

Keywords: SARS-CoV-2; COVID-19; Antibody levels; Viral load

1. Introduction

In late 2019, a novel coronavirus first identified in Wuhan, Hubei province, China, has spread rapidly worldwide, causing a global pandemic. The novel coronavirus was named SARS-CoV-2 and the disease it caused was called coronavirus disease 2019 (COVID-19). Effective human-to-human transmission of SARS-CoV-2 led to more than 200 million cases of COVID-19 reported globally until now, including more than 4,3 million deaths.

SARS-CoV-2 belongs to the lineage B of *Betacoronavirus* and shows genetic similarities with bat SARS-like CoV and human SARS-CoV [1]. Although SARS-CoV-2 have a much lower mortality rate than does SARS-CoV, its transmission among people is much more effective.

Patients with COVID-19 can present a wide range of clinical symptoms and disease severity. Many patients are asymptomatic or show only mild disease, while some present severe respiratory distress syndrome. Many risk factors for severe COVID-19 have been identified, including older age, male gender, obesity, comorbidities such as hypertension, type 2 diabetes mellitus and other chronic medical conditions [2-4].

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Relations between viral load and disease severity are not well studied, information is limited and results from such investigations are controversial. Information about kinetics of viral load and antibody levels is of great importance for reliable diagnosis, antiviral treatment and prognosis of COVID-19. At the same time, such data in scientific literature is scarce.

In this study, we investigated kinetics of viral load and antibody responses to SARS-CoV-2 in patients with COVID-19 and analysed the association with disease severity.

2. Material and methods

2.1. Patients and clinical samples

A total of 20 patients with COVID-19 were investigated. Eight of them were outpatients and 12 were hospitalized patients with more severe COVID-19. Diagnosis of COVID-19 of the patients has been previously laboratory confirmed by real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR). The patients were followed once per week within the first month after the onset and then at least once per month for the next 4 months. Pharyngeal swabs and serum samples were drawn from all patients. Laboratory investigations were performed at the National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria by ELISA and RT-PCR. Ethical approval for this study was obtained from Institutional review board at NCIPD (approval number 4/17.02.21). Written informed consent was obtained from all patients before the study. Severity of COVID-19 was interpreted as mild, moderate and severe according to the WHO guidance [5].

2.2. ELISA

Serum samples were tested for antibodies of the immunoglobulin classes IgA, IgM, and IgG against SARS-CoV-2 using ELISA tests (Euroimmun, Germany). Nucleocapsid protein of SARS-CoV-2 was used as antigen for detection of the specific antibodies. According to the manufacturer's instructions, results were calculated as ratio: OD of the sample/OD of the cut-off calibrator and were interpreted as positive for IgM/IgA/IgG when the ratio was ≥ 1,1.

2.3. Quantitative RT-PCR

Viral RNAs were extracted from nasopharyngeal swab samples using QIAmp Viral Mini Kit (Qiagen, Hilde, Germany) according to the manufacturer's instructions. SARS-CoV-2 viral load was quantified in a microplate by commercially available quantitative Realtime RT-PCR kit (Clonit Srl., Italy) that targets the N-region (nucleocapsid protein). Four RNA standards of known concentration were used to build a standard curve. The viral loads of SARS-CoV-2 represented the copy numbers of the N gene in the clinical samples from the patients.

3. Results

A total of 20 patients with laboratory confirmed COVID-19 diagnosis were enrolled in this study. Six of them (30%) presented with mild disease, 1 (5%) patient presented with mild to moderate disease, 10 (50%) patients were classified with moderately severe disease, 1 (5%) patient with moderate to severe disease, and 2 (10%) patients died because of the very severe COVID-19 disease (Table 1). Nine of the patients were male (45%) and eleven patients (55%) were female. The median age of the patients was 48.5 years.

Table 1 Baseline characteristics and disease severity in 20 patients with COVID-19

No.	Gender	Hospitalized	Age	Symptoms	Disease severity
1	M	No	34	fever 38-39^{0}C, cough, eye pain, headache, hyposmia, hypogeusia	mild
2	F	No	43	nasal congestion	mild
3	M	No	54	hyposmia, hypogeusia, subfebrile	mild
4	M	No	36	low-grade fever, sore throat	mild
5	F	Yes	43	cough, dyspnea, fever, headache, vomiting, pneumonia	moderate
6	M	Yes	44	fever, dyspnea, chest pain, pneumonia	moderate
7	F	No	25	fatigue, fever with chills, cough, sore throat	mild
8	F	Yes	64	fever 38^0 C, cough, malaise, pneumonia	moderate
9	M	Yes	46	fever, cough, myalgia, hemoptysis, dyspnea	moderate
10	F	Yes	66	cough, hemoptysis, malaise, dyspnea, pneumonia	moderate
11	M	Yes	61	subfebrile, dry cough, chest pain, dyspnea, pneumonia	moderate
12	M	Yes	35	bilateral pneumonia, dyspnea	moderate/severe
13	F	Yes	63	bilateral pneumonia, pleural effusion	moderate
14	F	Yes	63	bilateral pneumonia, respiratory failure	moderate
15	F	Yes	58	bilateral pneumonia, respiratory failure	severe/lethal
16	F	Yes	79	bilateral pneumonia, respiratory failure	severe/lethal
17	F	No	47	subfebrile, sore throat, rhinorrhea, myalgia, arthralgia	mild/moderate
18	F	No	35	hyposmia, fatigue	mild
19	M	No	38	fever, cough, pneumonia	moderate
20	M	Yes	35	bilateral pneumonia, pleural effusion	moderate

3.1. Viral load

Viral load kinetics in nasopharyngeal swabs of patients with COVID-19 is presented in Table 2. In all patients, the viral load gradually decreased over the time. Measurement of the viral load within first 1-4 days after the disease onset was possible for 11 patients. The majority of them (8/11 – 72,7%) presented high (10^6/ml) viral loads and 3/11 (27,3%) patients were with moderate viral loads (10^3-10^4/ml). Four of the patients with high viral load presented mild disease and the other four patients presented moderate disease and 3 of them were hospitalized. All patients with moderate viral load presented mild disease.

A week later, 7-11 days after the disease onset, the viral loads in most of the patients decreased significantly to low-grade viral loads (10^1-10^3/ml). However, two of the hospitalized patients (No. 8 and 11) who initially had very high (10^6/ml) viral loads, presented moderate viral loads (10^4/ml) and one of the patients who presented with mild disease had low decrease of the viral load (from 10^6 to 10^5) (No.17). Hospitalized patients, who were tested for the first time 7-11 days after the onset, showed low-grade viral loads (10^1-10^3/ml).

Two weeks after the disease onset, all patients had very low viral loads (10^0-10^2/ml). Maximal duration of detectable viral load was 22 days and it was registered in only one patient.

Table 2 Kinetics of viral load in patients with COVID-19

Patient No.	Days after onset of COVID-19									
	1-4 days		7-11 days		days		22-24 days		> 30 days	
	Ct	viral load	Ct	viral load	Ct	viral load	Ct	viral load	Ct	viral load
1	16	$1,22x10^6$	30	$2,56x10^2$	35	$1,73x10^1$	39	9,93	neg.	no
2	17	$2,22\ x10^6$	18	$3,90x10^5$	39	5,75	neg.	no		
3	28	$1,65x10^3$	36	$1,59x10^1$	39	2,39	neg.	no		
4	21	$8,93x10^4$	28	$4,62x10^2$	neg.	no				
5	n.d.	n.d.	25	$9,44x10^3$	28	$7,58x10^2$	neg	no		
6	n.d.	n.d.	n.d.	n.d.	31	$6,55x10^1$	neg	no		
7	17	$1,74x10^6$	26	$7,33x10^2$	33	$2,44x10^1$	neg	no		
8	15	$2,14x10^6$	24	$1,93x10^4$	28	$5,43x10^2$	neg	no		
9	n.d.	n.d.	39	$2,10x10^1$	neg.	no				
10	n.d.	n.d.	28	$8,92x10^2$	neg.	no				
11	15	$2,39x10^6$	22	$3,58x10^4$	33	8,35				
12	n.d.	n.d.	35	$5,8x10^1$	neg	no				
13	n.d.	n.d.	neg	no						
14	n.d.	n.d.	31	$6,95x10^1$	neg	no				
15	n.d.	n.d.	neg	no						
16	n.d.	n.d.	25	$3,58x10^3$	n.d.	n.d.				
17	12	$1,24x10^6$	20	$1,48x10^3$	neg.	no				
18	29	$1,15x10^2$	40	0.,084	neg.	no				
19	17	$1,11x10^6$	31	$3,13x10^2$	neg.	no				
20	22	$1,08x10^5$	29	$1,40x10^3$	neg.	no				

3.2. Antibody levels

Dynamics of serum concentrations of SARS-CoV-2-specific IgA, IgM and IgG antibodies in patients with COVID-19 are shown in Fig. 1-6.

The vast majority of patients with mild disease were seronegative 1-4 days after the disease onset. In fact, IgA antibodies were detected in only 1 of 7 patients, IgM in none and IgG antibodies in 2 of 7 patients with mild disease. However, most of the patients with moderately severe disease had specific antibodies 1-4 days after the onset. IgA antibodies were detected in 8 of 11 patients, IgM antibodies in 4 of 11 and IgG antibodies in 9 of 11 patients with moderate disease.

One week after the onset, seroconversion of IgA and IgG antibodies was usually detected in mild cases: IgA antibodies were detected in all 7 patients, IgM in 2 and IgG in 2 of the 7 patients with mild disease. At the same time, in patients with moderately severe disease, IgA antibodies were detected in all 11 patients, IgM was found in 9 patients and IgG in all 11 patients.

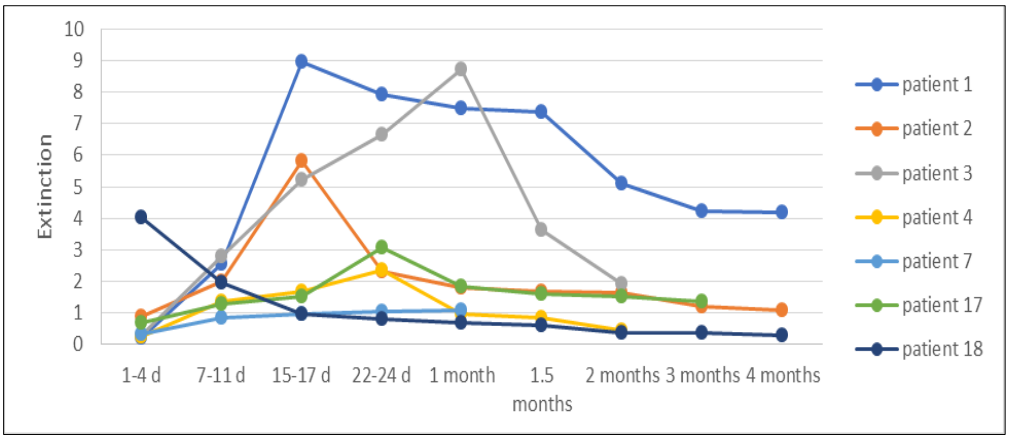

Figure 1 IgA antibody level kinetics in mild COVID-19 cases

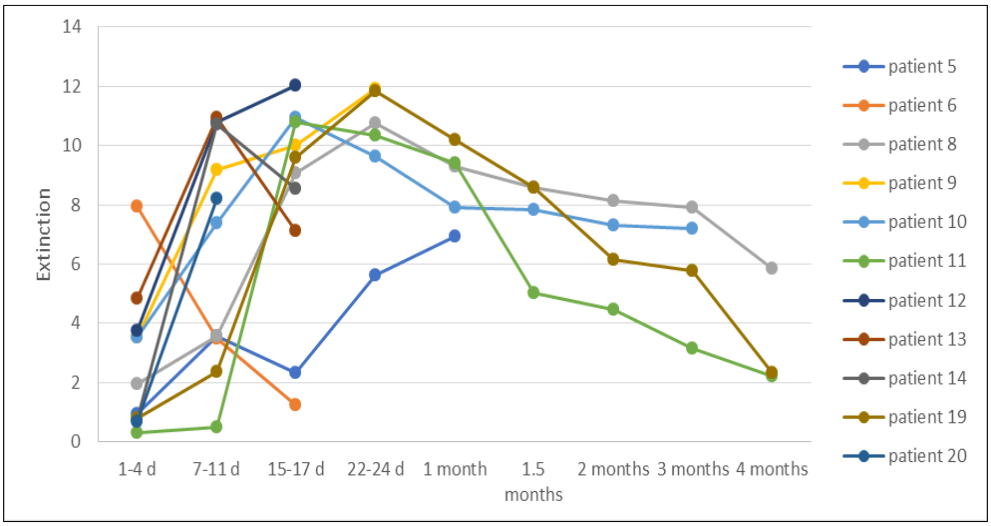

Figure 2 IgA antibody level kinetics in moderately severe COVID-19 cases

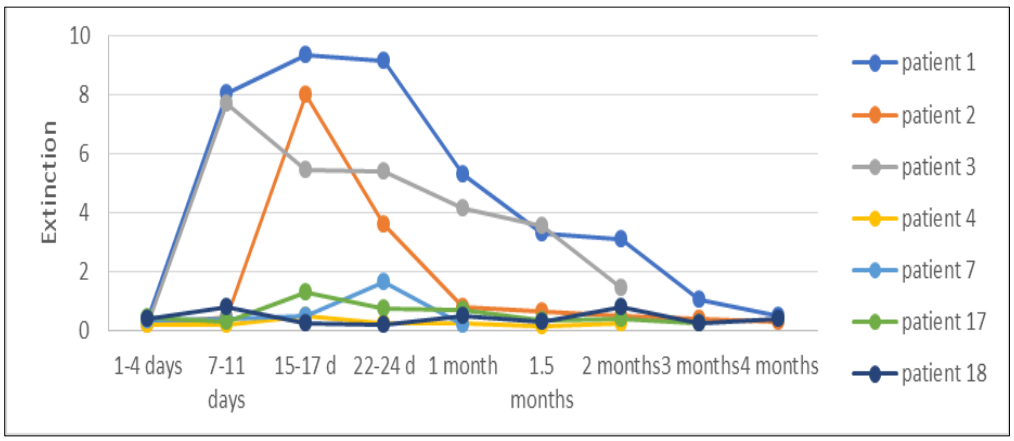

Figure 3 IgM antibody level kinetics in mild COVID-19 cases

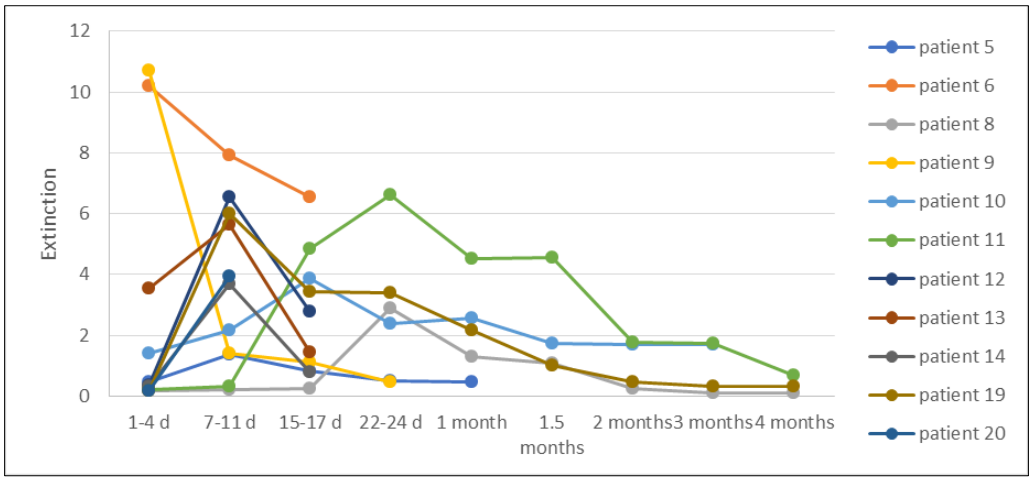

Figure 4 IgM antibody level kinetics in moderately severe COVID-19 cases

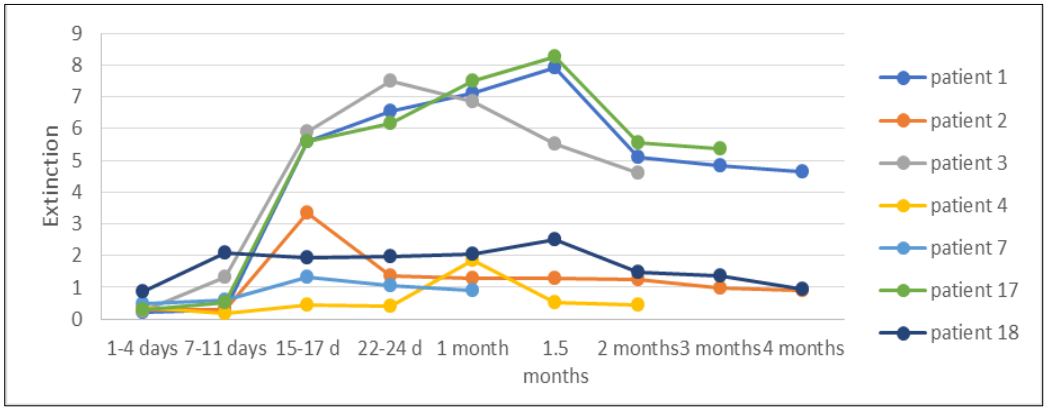

Figure 5 IgG antibody level kinetics in mild severe COVID-19 cases

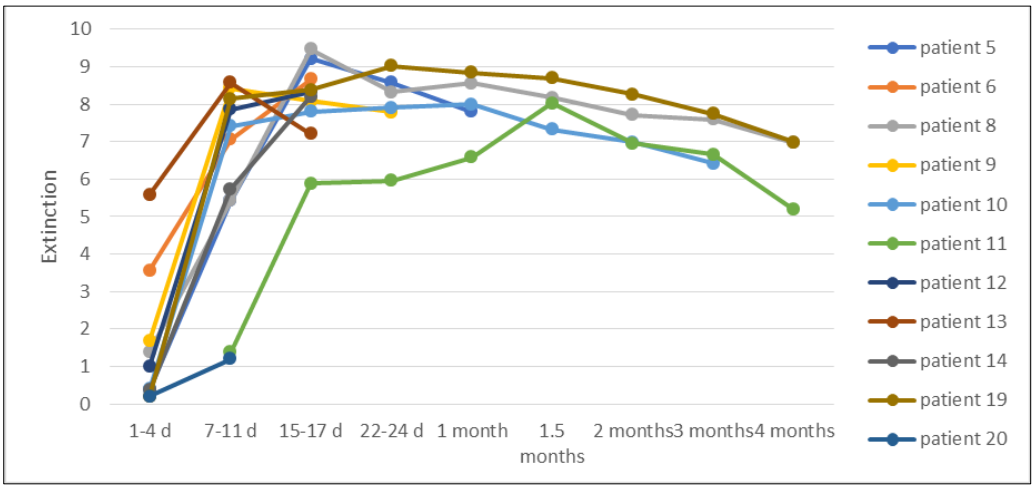

Figure 6 IgG antibody level kinetics in moderately severe COVID-19 cases

Two weeks after the onset, specific antibodies started to decrease gradually. One month after the onset, IgA antibodies were detected in 4 of 7 patients with mild disease and in all 5 patients with moderate disease who were followed; IgM

antibodies were detected in 2 of the 7 patients with mild disease and in 4 of 5 patients with moderate disease; IgG antibodies were found in 6 of 7 patients with mild disease and in all 5 followed patients with moderate disease.

Four months after the onset, all patients were seronegative for IgM antibodies, IgA antibodies were detected in 1 of 5 patients with mild disease and in all 4 followed patients with moderate disease, IgG antibodies were found in 3 of 5 patients with mild disease and in all 4 followed patients with moderate disease.

Levels of IgA, IgM, and IgG antibodies increased earlier to higher levels and were present for longer time in patients with more severe manifestations of COVID-19 than in those with mild disease. The data presented showed strong relation of disease severity and antibody kinetics.

4. Discussion

We analysed the kinetics of viral loads of SARS-CoV-2 and IgM, IgA and IgG antibody levels of patients with COVID-19. For vast majority of the patients, the viral loads were at their highest levels at presentation and then declined. Despite development of specific antibody response 7-11 days after the onset of COVID-19, we still detected SARS-CoV-2 RNA in nasopharyngeal swabs of most of the patients. In one patient, we detected viral RNA even 20 days after the onset. Based solely on the presented study, we did not find significant relation between viral loads and severity of the clinical presentation of the disease.

The peak viral load of SARS-CoV-2 was at the onset of the disease, in the first 1-4 days. There was no direct link between viral load and the severity of COVID-19: mild and severe cases started with a high viral load. There was a relationship between the time from the onset of the disease and the viral load: the highest viral load was in the first days and then gradually decreased.

Zou et al also found a high viral load in the first days after the symptom onset [6]. In fact, this study was based not on exact viral loads but on cycle threshold values. Nevertheless, the study also has shown high viral loads on admission. This fact suggests that SARS-CoV-2 can be very efficiently transmitted in the first days after the symptoms onset even if the disease is mild. Higher viral load before or immediately after the symptom onset is described for SARS-CoV-2, when the peak viral load of SARS-CoV is detected 10 days from the symptom onset [6,7].

We found very high viral loads in patients with limited number of symptoms and no later progression of COVID-19. In contrast, numerous studies on SARS have demonstrated that a high viral load at presentation was associated with higher probability of death [8].

Earlier reports have shown that serum IgG antibodies rise earlier than IgM antibodies against SARS-CoV-2 [9-10]. Our investigations revealed that IgG antibody response was not earlier than IgM antibody response but IgG antibodies were detected in more patients than IgM antibodies. This discrepancy could be explained partially by insufficient sensitivity of IgM tests.

Investigating these variables, Kwon et al [11] concluded that viral load and antibody response were higher in patients with more severe COVID-19. Shi et al [12] found natural fluctuation of the viral load in the early stage of COVID-19 and showed that patients with pneumonia had higher viral loads than patients with mild disease. Fajnzylber et al [13] found that higher SARS-CoV-2 plasma viral load was associated with worse disease severity and increased risk of mortality.

Our investigations showed slower reduction in viral load and longer detection of SARS-CoV-2 virus, i.e. slower virus cleaning in patients with more severe COVID-19. Maltezou et al [14] showed that patients with high viral load more often developed symptomatic disease, had longer stay in intensive care unit and longer intubation period compared to patients with low viral load. According to Zheng et al [7], the median duration of SARS-CoV-2 in the respiratory samples is longer in patients with severe disease.

Interestingly, our investigations showed almost simultaneous appearance of IgA, IgM and IgG antibodies. In patients with moderately severe COVID-19, the antibodies of the three classes appeared in the first week after the onset of the disease. In patients with mild disease, the antibodies appeared in the second week after the onset. In addition, in moderately severe forms the antibodies reached higher levels and remained elevated for longer time than in milder forms. In some patients with mild forms, the antibodies increased significantly early in the course of the disease but their levels dropped rapidly. In moderately severe forms, the antibodies were significantly elevated for more than 4 months.

The viral load kinetics is important for guiding antiviral treatment and for the infection control. Knowledge on antibody kinetics is important for proper timing requests for serological tests as well as for interpretation of results of the serological assays. In cases of late presentations of patients, serological investigation is the sole one to support the diagnosis. Further investigations are needed to reveal correlations between viral load, antibody levels and clinical severity of COVID-19.

5. Conclusion

Our study showed that viral load was inversely related to serum antibody response. The peak viral load was reached at the onset and in the first days after the onset of the disease. In patients with moderately severe COVID-19, viral load showed slower reduction and was in detectable levels for longer time than in patients with mild disease. Similarly, antibody levels reached higher levels and for longer time in patients with moderately severe COVID-19 in comparison to patients with mild disease.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflicts of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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