

Factors Associated with the Duration of Viral Shedding and the Role of Lopinavir/Ritonavir in Patients with COVID-19

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2. Key words

World Health Organization; COVID 19; Epidemic

1. Abstract

1.1. **Objective:** Coronavirus disease 2019 (COVID-19) causes severe community and nosocomial outbreaks; we aimed to ascertain the duration of viral shedding through repeated nasal swabs in patients with COVID-19 and factors associated

1.2. **Design:** observational study

1.3. **Setting:** 750 bedded University Hospital

1.4. **Patients:** case-series including all consecutive patients obtaining a negative SARS-CoV-2 nasal swab twice in succession after a laboratory confirmed COVID 19 between 3rd March and 5th May

1.5. **Intervention:** analysis of factors associated with the duration of viral shedding and role of lopinavir/ritonavir

1.6. **Results:** older age and hospitalization were independent risk factors associated with duration of viral shedding; the administration of LPV/r does not seem to affect the duration of viral shedding.

1.7. **Conclusion:** we found a median viral shedding of 23 days in the whole population while a significantly shorter shedding was observed in untreated patients; hospitalization and older age correlate with the duration of viral shedding whereas the administration of Lopinavir/Ritonavir does not seem to be associated. As we need an effective treatment to cure and to decrease virus carriage duration COVID-19 patients, results of ongoing studies and randomized clinical trials are strongly awaited.

3. Introduction

The 2019 novel coronavirus (SARS-CoV-2) epidemic has been declared a public health emergency by the World Health Organization and has drawn global intensive attention [1]. The ongoing outbreak in Northern Italy is actually associated with considerable morbidity and mortality resulting in a severe burden for the health care system.

The clinical spectrum of infection appears to be wide, ranging from asymptomatic infection, mild upper respiratory illness to severe viral pneumonia with respiratory failure. moreover nor effective treatments or primary prophylaxis are actually available and several randomized trials are currently in progress [2].

The duration of virus replication is an important factor in assessing

the risk of transmission and guidance around the length of isolation. moreover it has important implications in hospital setting in terms of PPE used by health care operators, time employed for the management of patients and spaces dedicated [3].

Here, we report a retrospective study with the primary purpose to describe the temporal trends of viral shedding in COVID 19 and to describe factors associated with its duration.

4. Methods

This study was conducted in adherence to the tenets of the Declaration of Helsinki and its later amendments by Humanitas Clinical and Research Center, a 750 bedded university hospital in Lombardy.

We included all consecutive patients reaching a negative SARS-

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CoV-2 nasal swab twice in succession after a laboratory confirmed COVID 19 obtained in our hospital between 3rd March and 5th May. COVID 19 was confirmed by testing nasopharyngeal swab with a real-time reverse transcription-polymerase chain reaction (RT-PCR) assay.

All the patients in this study were symptomatic patients; patients with mild illness, as defined by COVID 19 disease severity scale on latest clinical guidelines enacted by WHO on May 27, 2020 were not hospitalized.

Clinical characteristics, treatments and outcome were obtained from electronic medical records.

Duration of viral RNA shedding was considered as the number of days from symptom onset to persistent negative detection of respiratory tract specimens; all subsequent specimens from the same patients were tested until two consecutive samples were negative defining the duration of shedding.

5. Statistical Analysis

Continuous variables were expressed as median with interquartile range (IQR). Categorical variables were expressed as number (%).

We employed Kaplan-Meier survival analysis to estimate the cumulative SARS-CoV-2 negativity rate stratified between patients with and without therapy; log-rank statistic to compare the difference of SARS-CoV-2 clearance.

Significant risk factors identified on univariate analyses were further analyzed by log-linear regression model ($\log Y_i = \alpha + \beta X_i + \epsilon_i$) to identify factors associated with SARS-CoV-2 shedding duration.

Logarithmic transformations are convenient means of transforming a highly skewed variable into approximately normal.

In the log-linear model, the literal interpretation of the estimated coefficient β is that a one-unit increase in X will produce an expected increase in log Y of β units. In terms of Y itself, this means that the expected value of Y is multiplied by e^β .

Each 1-unit increase in X multiplies the expected value of Y by e^β .

All statistical analyses were performed using STATA 15 and the p-value limit for statistical significance was set at $p < 0.05$.

6. Results

The study population included 168 consecutive patients with confirmed SARS-CoV-2 infection who achieved the viral clearance (negative nasal swab on two consecutive days) in the period from March 3rd to May 5th.

All the respiratory specimens tested were derived from nasal swabs.

Among 168 patients enrolled, 88/168 (52%) were male and median age was 47 years (IQR 39-60).

80/168 (47%) were hospitalized.

Lopinavir/ritonavir (LPV/r) was the most frequently administered antiviral regimen (69/72 treated); hydroxychloroquine (HCQ) was associated in 94% of cases.

Table 1 shows the main characteristics of the patients who were included in the final analysis and the differences between patients treated and untreated.

Table 1. Characteristic of patients with SARS-CoV-2 infection

Characteristic	All patients (n=168)	Therapy (n=72)	No therapy (n=96)
Median age (IQR) –years	47 (39-60,5)	59,5 (48-70)	42 (33-47,5)
15-24	8	0	8
25-34	18	0	18
35-44	41	9	32
45-54	34	13	21
55-64	29	20	9
65-74	25	21	4
75-84	10	9	1
95+	3	0	3
Female sex (%)	80	19	61
Median time to negativization (IQR) -days	23 (17-31)	30 (23-36)	21 (14-28)
Hospitalization (%)	80%	-	-
Lopinavir/ritonavir treatment (n)		69	-
Time between onset of symptoms and treatment Started \geq 7 days		48%	-

In our study the median duration of SARS-CoV-2 shedding in the whole population was 23 days (IQR 17-31).

Among hospitalized patients, the median hospital length of stay was 11,5 days.

A significantly longer lasting shedding was observed in treated patients in comparison with those untreated (median 30 days vs 21 days, $p < 0.01$) as shown in (Figure 1).

In the multivariate logistic model older age and hospitalization were independent risk factors associated with duration of viral shedding (Table 2).

Of the 168 patients included, 69 were administered with lopinavir/ritonavir treatment.

Patients receiving lopinavir/ritonavir were all hospitalized and were more likely to have a severe COVID 19.

In 48% of cases, LPV/r was initiated after 7 days from symptom onset.

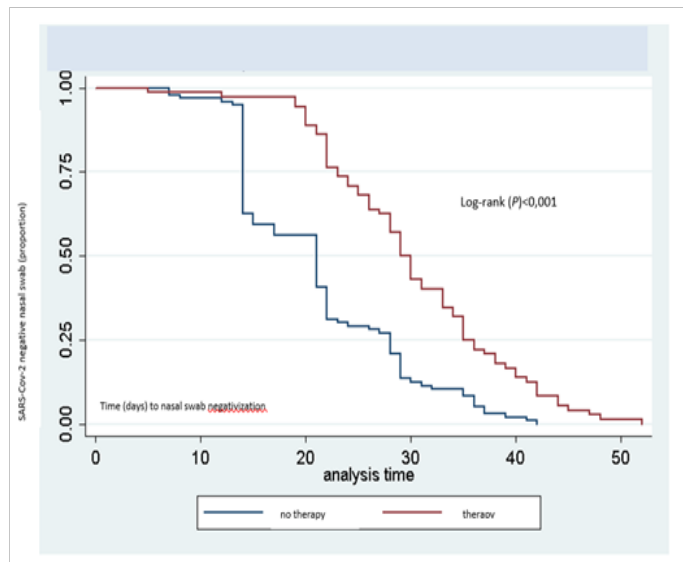


Figure 1. Cumulative SARS-CoV-2 RNA negativity rate stratified between treated and not treated patients

Table 2. Log linear analysis of factors associated with duration of SARS-CoV-2 RNA detection

Variable	Age	Coef	Stand err	95% CI		P	e ^{B^A}
	15-24 vs 35-44	-0,066	0,139	-0,340	0,209	0,637	0,94
	25-34 vs 35-44	-0,036	0,103	-0,238	0,167	0,728	0,96
	45-54 vs 35-44	0,097	0,084	-0,068	0,263	0,246	1,10
	55-64 vs 35-44	0,067	0,094	-0,118	0,253	0,473	1,07
	65-74 vs 35-44	0,180	0,103	-0,023	0,382	0,082	1,20
	75-84 vs 35-44	0,336	0,137	0,066	0,605	0,015*	1,40
	95+ vs 35-44	-0,137	0,214	-0,560	0,286	0,522	0,87
	Female sex	-0,030	0,061	-0,151	0,091	0,623	0,97
	Lopinavir/ritonavir yes vs no	-0,105	0,134	-0,371-0,160		0,434	0,90
	Hospitalization yes vs no	0,399	0,136	0,131-0,667		0,004*	1,49

In the multivariate logistic model the administration of LPV/r seems to not affect the duration of viral shedding.

7. Discussion

Viral shedding is commonly used as a proxy measure of the infectivity; hence the identification of the duration of viral shedding would be central to inform control policies and treatment strategies in patients with COVID 19.

Several studies have shown how SARS-CoV-2 spreads more rapidly than MERS-CoV and SARS-CoV; Zou et al [4]. demonstrated higher viral loads likewise in symptomatic and asymptomatic patients soon after symptom onset.

Moreover the viral shedding of patients infected with SARS-CoV-2 appears different from that seen in patients with SARS-CoV and resembles that of influenza [5].

Nevertheless the pattern of SARS-CoV-2 shedding during the course of disease and therapy hasn't been well characterized.

Wang et al [3]. reported a median duration of SARS-CoV-2 RNA shedding of up to 19,5 days; in another study including 191 cases a median detectable SARS CoV2 RNA of 20 day was reported in survivors and a persisting duration of shedding in non-survivors [6].

Our results demonstrate a median viral shedding of 23 days in the whole population while a significantly shorter shedding was observed in untreated patient, arguably without therapy for a less severe disease.

Moreover, in the multivariable model hospitalization and older age are independently associated with the duration of viral shedding.

As hospitalization can be considered as a proxy of the severity of disease, this result is consistent with studies that demonstrated how critically ill patients have longer lasting viral shedding [7].

Nevertheless, as regards as the relationship between viral shedding and severity of disease, further studies are needed to ascertain whether critically ill patients have longer viral shedding as literature data appear to be conflicting [8].

On the contrary the observed association between age and shedding contributes to existing literature data by addressing the interplay between shedding and ageing; among this finding a reasonable explanation can be a less competent innate and adaptive immune system against the virus.

It has been demonstrated that lopinavir/ritonavir, a human immunodeficiency virus 1 protease inhibitor, is effective in patients infected with SARS-CoV and in animal models for MERS-CoV [9].

In a recent randomized controlled trial including patients with COVID 19, Cao et al [6]. showed that lopinavir/ritonavir alone was similar to placebo in reducing viral load despite some improvements in symptoms; nevertheless in a post hoc subgroup analysis the early administration of lopinavir/ritonavir was associated with a reduced mortality.

In our study we didn't observed any association between the administration of lopinavir/ritonavir and the lasting of viral shedding.

Some other studies observe how a combination antiviral treatment administrated early from symptom onset can be associated with a shorter shedding and viral load negativization [10].

Finally further studies are awaited to ascertain the role of antivirals in reducing the viral shedding.

Our study has some limitations; first we reported only viral shedding measurement without assessment of the infectivity. It is not known how shedding of viral RNA correlates with shedding of infectious virus and further studies are warranted to ascertain whether the patients are shedding live virus.

Second the estimated duration of viral shedding is limited by the

frequency of respiratory specimen collection and relatively low positive sensitivity of SARS-CoV-2 detection through nasal swabs.

Finally, our findings can be limited by sample size and the lack of viral load assessment.

In summary we found that hospitalization and older age correlate with duration of viral shedding whereas administration of lopinavir/ritonavir seems not to be associated.

As we need an effective treatment to cure COVID-19 patients and to decrease virus carriage duration, results of ongoing studies and randomized clinical trials to determine the effectiveness of treatment in patients with COVID 19 are strongly awaited.

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