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

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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 seems 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.

2. Key words

World Health Organization; COVID 19; Epidemic

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 immense global attention. The ongoing outbreak in Northern Italy is presently 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. To date, neither effective treatments nor primary prophylaxis are available although several randomized trials are currently in progress.

The duration of virus replication is an important factor in assessing the risk of transmission and guidance around the length of isolation. This also has important implications in hospital settings in terms of PPE used by health care operators, time employed for the management of patients and dedicated areas.

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

4. Methods

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

In our study we included all consecutive patients obtaining a neg-

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

All the patients in this study were symptomatic patients; no hospitalization occurred for patients with mild illness, as defined by COVID 19 disease severity scale in the latest clinical guidelines published by WHO on 27th May, 2020.

For patients with a diagnosed mild disease and no need for medical support and hospitalization, domiciliary quarantine was applied until negativization.

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

The 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 and 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 Yi = α + βXi + εi) 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. All the patients had achieved viral clearance (negative nasal swab on two consecutive days) in the period between 3rd March and 5th May 2020.

All the respiratory specimens tested were derived from nasal swabs. Among the 168 enrolled patients, 88/168 (52%) were male and the median age was 47 years (IQR 39-60) with 80/168 (47%) being hospitalized.

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

The main characteristics of the patients who were included in the final analysis and the differences between patients treated and untreated is shown in (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Characteristic of patients with SARS-CoV-2 infection</th>
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<tbody>
<tr>
<td>Characteristic</td>
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<td>--------------------------------------------------------------------------------</td>
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<tr>
<td>Median age (IQR) – years</td>
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<td>Female sex (%)</td>
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<td>Median time to negativization (IQR) - days</td>
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<td>Hospitalization (%)</td>
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<td>Lopinavir/ritonavir treatment (%)</td>
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<td>Time between onset of symptoms and treatment Started ≥ 7 days</td>
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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).

![Kaplan-Meier survival estimates](image-url)

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

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 in the study, 69 were administered with Lopinavir/Ritonavir treatment. These latter patients were all hospitalized and were more likely to have severe COVID 19. In 48% of cases, LPV/r was initiated after 7 days from symptom onset. In the multivariate logistic model the administration of LPV/r does not seem to affect the duration of viral shedding.

7. Conclusion

Viral shedding is commonly used as a proxy measure of infectivity; hence the identification of the duration of viral shedding should 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. demonstrated higher viral loads likewise in symptomatic and asymptomatic patients soon after symptom onset. Furthermore, 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. So far, the pattern of SARS-CoV-2 shedding during the course of disease and therapy has not been well defined. Wang et al. reported a median duration of SARS-CoV-2 RNA shedding of up to 19.5 days and in another study of 191 cases a median detectable SARS CoV2 RNA of 20 day was reported in survivors and a persisting duration of shedding in non-survivors.

Our results demonstrate a median viral shedding of 23 days in the whole population while a significantly shorter shedding was observed in untreated patients, arguably without therapy for a less severe form of the disease. Additionally, 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 have demonstrated how critically ill patients have longer lasting viral shedding. However, concerning 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. The observed association between age and shedding contributes to existing literature by addressing the interplay between shedding and ageing; this finding may indicate 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. In a recent randomized controlled trial including patients with COVID 19, Cao et al. showed that Lopinavir/Ritonavir alone was similar to a 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 did not observe any association between the administration of Lopinavir/Ritonavir and the duration of viral shedding. Some other studies have observed how a combination of antiviral treatment administrated from symptom onset can be associated with a shorter shedding (Hung et al) [10] and viral load negativization (Deng et al) Finally, further studies are awaited to ascertain the role of antivirals in reducing the viral shedding.

Our study has some limitations. Firstly, we reported only viral shedding measurements without assessment of 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. Secondly, 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 may have been limited by sample size and the lack of viral load assessment.

In conclusion, we found that hospitalization and older age correlate with the duration of viral shedding whereas the administration of Lopinavir/Ritonavir does not seems to be associated.

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

References


