



ORIGINAL RESEARCH

# Prevalence and Clinical Significance of Persistent Viral Shedding in Hospitalized Adult Patients with SARS-CoV-2 Infection: A Prospective Observational Study

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## ABSTRACT

**Background:** The goal of this study was to investigate the prevalence and factors associated with persistent viral shedding (PVS) in hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

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**Methods:** This was a prospective observational study including all consecutive adults hospitalized with SARS-CoV-2 infection. When the first nasopharyngeal swab was positive for SARS-CoV-2 RNA (day 0), additional samples were obtained on days + 3, + 5, + 7 and then once every 7 days until virus detection was negative. PVS was defined as the duration of shedding of at least 21 days after diagnosis. The primary endpoint of this study was the prevalence of PVS.

**Results:** Data were obtained regarding 121 consecutive hospitalized patients with SARS-

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CoV-2 infection (median age 66 years, male sex 65.3%). Overall, the prevalence of PVS was 38% (46/121 patients). According to univariate analysis, factors associated with PVS were immunosuppression (6.7% vs 21.7%,  $p = 0.02$ ), increased interleukin-6 (IL-6) levels ( $\geq 35$  ng/ml) at the time of diagnosis (43.4% vs 67.3%,  $p = 0.02$ ), time from onset of symptoms to diagnosis (median days 7.0 vs 3.5,  $p = 0.001$ ), intensive care unit admission (22.7% vs 43.5%,  $p = 0.02$ ), and need for invasive mechanical ventilation (20.0% vs 41.3%,  $p = 0.01$ ). The multivariate analysis indicated that immunosuppression, increased IL-6 levels at the time of diagnosis, time from onset of symptoms to diagnosis, and need for mechanical ventilation were independent factors associated with PVS. **Conclusions:** PVS was detected in up to 38% of hospitalized patients with SARS-CoV-2 infection and was strongly associated with immunosuppression, increased IL-6 levels, and the need for mechanical ventilation.

**Keywords:** COVID-19; IL-6; Outcome; SARS-CoV-2; Viral shedding

### Key Summary Points

SARS-CoV-2 viral shedding usually occurs within 21 days but several patients experience persistent virus shedding (PVS).

We found that 38% of hospitalized adult patients with SARS-CoV-2 infection had persistent viral shedding.

Immunosuppression and increased IL-6 levels at the time of diagnosis slow viral clearance and are associated with longer hospital stay and poorer outcome.

Future investigations analyzing the potential for transmission in patients with PVS are required.

## DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to <https://doi.org/10.6084/m9.figshare.13148453>.

## INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus has recently emerged as a new cause of acute respiratory disease [1–3], whose clinical spectrum encompasses asymptomatic infection, mild upper respiratory tract illness, and severe pneumonia with respiratory failure and even death [4–7].

Viral clearance in the respiratory tract usually occurs within 21 days [8, 9]. However, a variable proportion of patients with SARS-CoV-2 infection may experience persistent virus shedding (PVS) lasting up to 60–80 days after diagnosis [10–12]. Unfortunately, our understanding of why some patients with SARS-CoV-2 infection do or do not present with PVS has not been fully investigated and PVS remains an entity of uncertain clinical significance [13–19]. Literature on this topic is scarce and difficult to interpret because of the heterogeneity of definitions proposed [15–19] or different specimens collected for RNA detection (upper versus lower respiratory tract samples) [15].

In this study, we analyzed viral shedding data of adult patients with SARS-CoV-2 infection admitted to our hospital. Our aims were to investigate (1) the prevalence of PVS and (2) the association between PVS and patients' underlying diseases, interleukin-6 (IL-6) levels at the time of diagnosis, and clinical outcome. We hypothesized that immunosuppressed patients with marked cytokine activation would be more likely to present with persistent SARS-CoV-2 RNA shedding.

## METHODS

### Patients and Study Design

This was a prospective observational study of patients with laboratory-confirmed SARS-CoV-2 infection who were consecutively admitted to San Martino Policlinico Hospital (Genoa, Italy) from February 25, 2020 through March 25, 2020. Our institution is a 1200-bed teaching hospital with a full range of clinical services and attending an urban population of approximately 400,000 persons. The study was carried out in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of Liguria Region (N. CER Liguria 114/2020—ID 10420). All patients provided verbal informed consent because of isolation precautions for performing nasopharyngeal swab according to the local protocol approved by hospital authorities, for data collection and for participation in the study.

### Test Performed

As soon as the first nasopharyngeal swab was positive for SARS-CoV-2 RNA (day 0), designated research doctors obtained the patient's consent and managed each case according to routine clinical care, until death or hospital discharge. Consecutive nasopharyngeal swabs for SARS-CoV-2 real-time reverse transcriptase (RT)-PCR were obtained at day + 3, day + 5, day + 7, and then once every 7 days until viral detection was negative. If a first nasopharyngeal swab was negative, a second one was repeated after 24 h in order to confirm its negativity and, potentially, discontinue droplet precautions in patients with clinical resolution of the symptoms. Additionally, research doctors requested routine coagulation, and biochemical blood tests at day 0 and when clinically indicated.

### Exclusion Criteria

Exclusion criteria included (1) patients aged less than 18 years; (2) patients with positive nasopharyngeal swabs who were discharged

early (less than 21 days from diagnosis) into fiduciary isolation (also known as self-isolation); (3) death within the first 21 days after diagnosis without evidence of SARS-CoV-2 RNA clearance; (4) missing virologic data at any study time point.

### Standard of Care and Treatment Procedures

During the study period, patients with suspected SARS-CoV-2 infection were managed and treated according to a local protocol. Briefly, nasopharyngeal swabs were taken in all symptomatic patients with suspected SARS-CoV-2 infection. Patients were only admitted to the hospital if they presented with acute respiratory insufficiency ( $\text{PaO}_2 < 60$  mmHg at rest in ambient air) or if the exacerbation of their underlying disease or severe symptoms were considered unmanageable at home. According to local guidelines, short-term antibiotic coverage and hydroxychloroquine 400 mg bid p.o. were recommended to all patients. Darunavir/ritonavir 800/100 once daily for 10 days was also recommended until March 24. Thereafter, the combination darunavir/ritonavir was withdrawn on the basis of published data regarding possible lack of efficacy of protease inhibitors for treating SARS-CoV-2 infections [20]. Since March 11 a single intravenous infusion of tocilizumab (dose 8 mg/kg) was also considered in the case of severe COVID-19 pneumonia. Moreover, following an internal review of risks and benefits of steroid treatment in patients with severe COVID-19, since March 19 intravenous administration of methylprednisolone (1 mg/kg for 5 days, followed by 0.5 mg/kg for another 5 days) was included in the protocol and administered in the emergency department in patients with severe pneumonia due to SARS-CoV-2.

### Data Collected and Definitions

Detailed information regarding data collected and definitions used in the present study are provided in the supplementary material. Briefly, clinical data were recorded with the use of a pre-

established protocol that included the following: demographics; underlying disease; clinical, laboratory and radiological findings at presentation; data on treatment; SARS-CoV-2-related complications; and outcome.

The duration of shedding was calculated on the basis of the time elapsed between the first positive nasopharyngeal swab and the first of two consecutive negative swabs taken 24 h apart. Persistent viral shedding was a priori defined as the duration of shedding of at least 21 days, which was recently described to be the median shedding duration of SARS-CoV-2 in an unselected group of patients [8, 9, 14].

Immunosuppression was defined as the presence of either (1) HIV infection and/or (2) hematological malignancy and/or (3) chronic treatment with immunosuppressant drugs and/or (4) a solid organ transplantation.

### Main Outcomes Measure

The primary endpoint was the PVS among our study population including hospitalized adult patients with SARS-CoV-2.

The secondary endpoint was to investigate the association between PVS and patients' underlying diseases (hypertension, cardiovascular disease, immunosuppression, diabetes mellitus, chronic kidney disease, cerebrovascular disease, chronic obstructive lung disease, solid tumor), inflammatory biomarkers at the time of diagnosis (D-dimer, C-reactive protein (CRP), and IL-6 levels), and clinical outcome (need for non-invasive respiratory support or invasive mechanical ventilation, intensive care unit (ICU) admission, development of septic shock, and need for continuous renal replacement therapy).

### Sample Processing and SARS-Cov-2 Virus Detection

SARS-CoV-2 RNA detection in nasopharyngeal swab was performed using RT-PCR targeted at open reading frame 1ab (ORF 1ab) and nucleocapsid protein (N) genes. RNA was extracted from the samples and then underwent real-time RT-PCR with SARS-CoV-2-specific primers and

probes. A cycle threshold (Ct) value of less than 37 was defined as a positive test, and a Ct value of 40 or more was considered as a negative test. An equivocal result, defined as a Ct value between 37 and 40, required confirmation by retesting. If the repeated Ct value was less than 40 and an obvious peak was observed, or if the repeated Ct value was less than 37, the result was deemed positive.

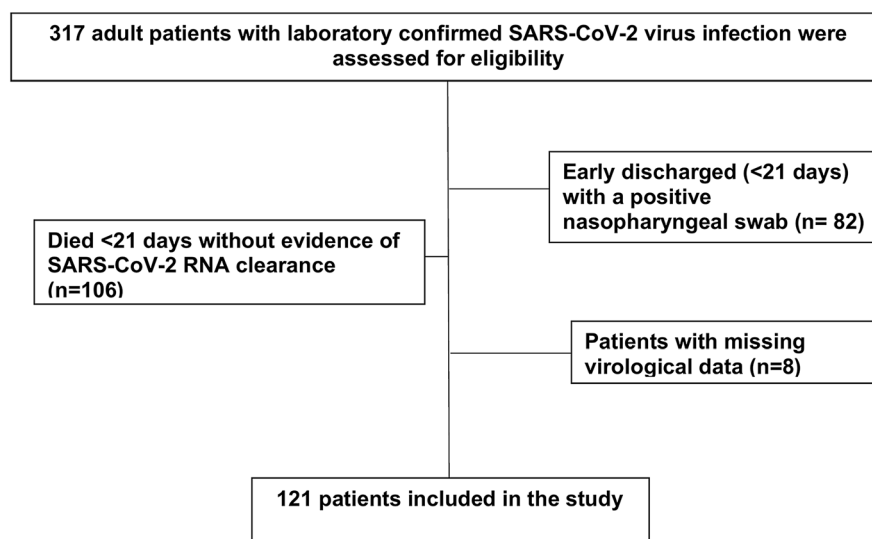
### Statistical Analysis

No statistical sample size calculation was done, and sample size was equal to the number of hospitalized patients with SARS-CoV-2 infection fulfilling inclusion criteria. Descriptive data were reported as median with interquartile ranges (IQR) or count and percentage, as appropriate.

Univariable and multivariate backward stepwise regression analyses were performed to evaluate the association between PVS and host factors and clinical outcome. Variables with  $p < 0.10$  in the univariate models were candidates for multivariate analysis. Two-sided  $p$  values of 0.05 or less were considered as statistically significant. Data were retrieved from an online database for anonymous and automatic data collection [21]. The analyses were performed using SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

During the study period, 317 patients with SARS-CoV-2 infection were admitted to our hospital. Of those, 106 (33.4%) died within the first 21 days after diagnosis without evidence of SARS-CoV-2 RNA clearance, 82 (25.8%) were discharged early (less than 21 days from diagnosis) into fiduciary isolation and 8 (2.5%) had missing virological data. The remaining 121 patients fulfilled the inclusion criteria and were included the present study (Fig. 1). Clinical characteristics, treatments, and main outcomes of our study population are shown in Table 1.



**Fig. 1** Flow diagram of hospitalized patients with confirmed SARS-CoV-2 infection included in this study

### Cumulative Incidence and Factors Associated with PVS

Overall, the definition of PVS (21 days or more) was fulfilled by 46/121 patients (38.0%), of whom 33 (27.3%), 15 (12.3%), and 5 (4.1%) continued to present viral shedding on day 28, day 35, and day 42, respectively. The cumulative incidence of patients with positive SARS-CoV-2 PCR at different time points is shown in Fig. 2.

Comparison between patients with PVS and those without PVS is shown in Table 2. According to univariate analysis, factors associated with PVS were immunosuppression (6.7% vs 21.7%,  $p = 0.02$ ), increased IL-6 levels at the time of diagnosis (43.4% vs 67.3%,  $p = 0.02$ ), time from onset of symptoms to diagnosis (median days 7.0 vs 3.5,  $p = 0.001$ ), ICU admission (22.7% vs 43.5%,  $p = 0.02$ ), and need for invasive mechanical ventilation (20.0% vs 41.3%,  $p = 0.013$ ). PVS was not associated with antiviral treatment and anti-inflammatory therapy (corticosteroids or tocilizumab). The multivariate analysis confirmed immunosuppression, increased IL-6 levels at the time of diagnosis, time from onset of symptoms to diagnosis, and need for invasive mechanical ventilation as independent factors associated with PVS.

### DISCUSSION

In our study population of hospitalized patients with SARS-CoV-2 infection, we found that persistent viral shedding is common and associated with immunosuppression and increased IL-6 at the time of diagnosis, as well as time from onset of symptoms to diagnosis and the need for mechanical ventilation.

The present study analyzed a large database of prospectively collected data concerning hospitalized patients with SARS-CoV-2 infection in a university tertiary care hospital in Italy. To our knowledge, it is also the largest European prospective observational study analyzing SARS-CoV-2 RNA shedding using homogenous specimens (nasopharyngeal swabs), providing a unique opportunity to understand the clinical significance of viral shedding in these patients. Data were collected according to daily clinical practice, making our findings representative for the current management of patients with infections due to SARS-CoV-2.

The lack of a commonly accepted definition makes it difficult to address the issue of PVS in SARS-CoV-2 infection [13–16]. Some authors considered PVS as the detection of SARS-CoV-2 RNA more than 15 days after symptoms [15]. Others required a longer period of time (e.g.,

**Table 1** Clinical characteristics, treatments, and outcomes of the 121 patients with SARS-CoV-2 infection included in the study

Characteristics <sup>a</sup>	Total ( <i>N</i> = 121)
Age, years (median, IQR) <sup>b</sup>	66 (55–76)
Sex, male	79 (65.3)
Underlying disease <sup>b</sup>	
Hypertension	55 (45.5)
Cardiovascular disease	16 (13.2)
Immunosuppression	15 (12.4)
Diabetes mellitus	15 (12.4)
Chronic kidney disease	8 (6.6)
Cerebrovascular disease	6 (5.0)
Chronic obstructive lung disease	5 (4.1)
Signs and symptoms <sup>b</sup>	
Fever ( <i>T</i> <sub>c</sub> > 37.3 °C)	111 (91.7)
Shortness of breath	63 (52.1)
Dry cough	61 (50.4)
Asthenia	27 (22.3)
Diarrhea	10 (8.3)
Nausea and vomiting	9 (7.4)
Mental confusion	7 (5.8)
Laboratory findings <sup>b</sup>	
Lymphopenia	107 (88.4)
Hypoxemic respiratory failure	77 (63.6)
AST > 40 UI/L	55 (45.4)
ALT > 40 UI/L	44 (36.3)
Thrombocytopenia	35 (28.9)
Leukopenia	21 (17.3)
Inflammatory markers <sup>b</sup>	
C-reactive protein > 5 mg/L	109 (90.1)
D-dimer > 500 µg/L	102 (84.3)
IL-6 ≥ 35 ng/L	64 (52.9)
Radiologic findings <sup>b,c</sup>	

**Table 1** continued

Characteristics <sup>a</sup>	Total ( <i>N</i> = 121)
Interstitial pattern	57/117 (48.7)
Bilateral consolidation	50/117 (42.7)
Monolateral consolidation	31/117 (26.5)
Absence of lesions	14/117 (12.0)
Pleural effusion	3/117 (2.5)
Time from onset of symptoms to diagnosis (median, IQR)	6.0 (3.0–8.0)
Treatments	
Hydroxychloroquine	109 (91.0)
Darunavir/ritonavir	72 (59.5)
Corticosteroids	64 (52.9)
Tocilizumab	33 (27.3)
Oseltamivir	15 (12.4)
Complications during hospitalization	
Non-invasive respiratory support	66 (54.5)
ICU admission	37 (30.6)
Invasive mechanical ventilation	34 (28.1)
Septic shock	10 (8.3)
Continuous renal replacement therapy	7 (5.8)
All-cause in hospital mortality	12 (9.9)

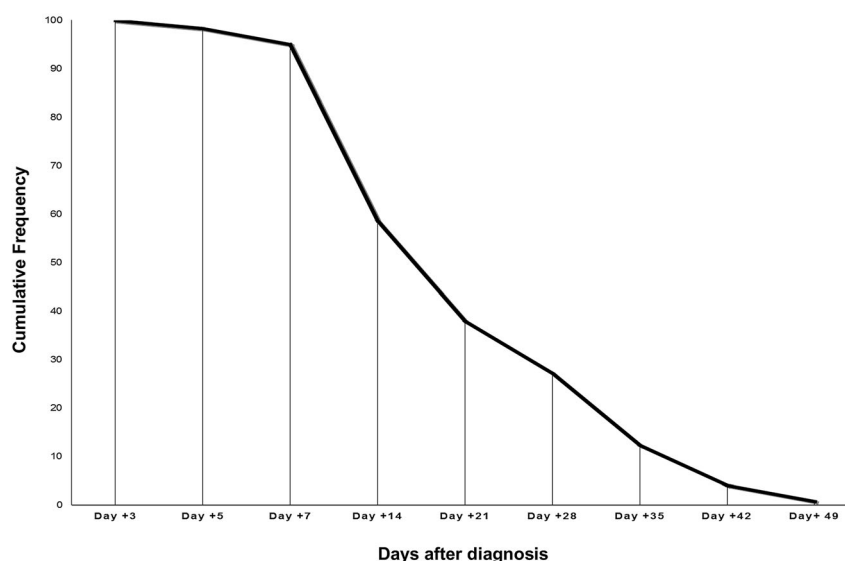
*AKI* acute kidney injury, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ICU* intensive care unit, *IQR* interquartile range

<sup>a</sup> Unless otherwise specified, data are presented as *n* (% of the total = 121)

<sup>b</sup> Data were collected at the time of diagnosis of SARS-CoV-2 infection

<sup>c</sup> Chest CT was performed in 117 out of 121 patients at the time of SARS-CoV-2 infection





**Fig. 2** Cumulative frequency of patients with positive SARS-CoV-2 RNA at different time points (percentage)

more than 24 days) [16]. Our definition of PVS is more consistent with the recent literature reporting SARS-CoV-2 RNA detection for a median of 21 days after symptom onset [8, 9, 14]. Using this specific time point, we found that up to 38% of hospitalized patients with SARS-CoV-2 infection experienced PVS, which is within the 32.7–48.2% range of PVS reported in other series [15, 16]. However, we are not sure whether PVS means that virus is viable and can be transmitted. Indeed, SARS-CoV-2 infectiousness may significantly decline 8–18 days after symptoms onset, as suggested by previous studies in which live virus could no longer be cultured despite concomitant positive PCR results [12, 22]. Future investigations analyzing the potential for transmission in patients with PVS are required.

The clinical significance of PVS in patients with SARS-CoV-2 remains unclear. Previous studies related PVS to male sex [15], older age [13, 14, 16], or chronic comorbidities [16]. In our study population, we did not find any correlation between PVS and age or sex, although immunosuppression significantly increased the risk of PVS sevenfold ( $p = 0.019$ ). This observation may highlight individuals who need to be closely monitored or on whom treatment interventions should be focused in the future.

Interestingly, we found a higher probability of PVS among patients with increased levels of IL-6 at the time of diagnosis. This result is consistent with recent experimental models suggesting that increased IL-6 levels during antiviral immune responses can negatively impact the lytic activity of cytotoxic T lymphocytes [23], thus promoting viral persistence. Ours is the first study to show this association in SARS-CoV-2 infection. This finding may point to the complex role of immunomodulators in the treatment of patients with SARS-CoV-2 infection [24].

We also found that PVS was mainly observed in patients with a more severe clinical course of SARS-CoV-2 infection. This was also so in previous studies, with the need for mechanical ventilation being 9.8 times more frequently in patients with persistent viral shedding when compared with those presenting faster conversion [15].

We believe that our results represent a significant step forward in better management of patients with SARS-CoV-2 infection. As a matter of fact, a more stringent approach to infection control may be necessary for immunosuppressed patients, including strict droplet precautions and isolation for an extended period of time than that currently recommended (14 day)

**Table 2** Univariate and multivariate analysis of factors associated with persistent viral shedding in patients admitted to the hospital with SARS-CoV-2 infection

Characteristics	Patients without PVS (N = 75)	Patients with PVS (N = 46)	p	OR (95% CI)	p
Age, years (median, IQR) <sup>a</sup>	65 (54–76)	67.5 (60.0–76.3)	0.23	–	–
Sex, male <sup>a</sup>	46 (61.3)	33 (71.7)	0.32	–	–
Underlying disease <sup>a</sup>					<b>0.019</b>
Hypertension	37 (49.3)	18 (39.1)	0.34		
Cardiovascular disease	7 (9.3)	9 (19.6)	0.16		
Diabetes mellitus	7 (9.3)	8 (17.4)	0.25		
Immunosuppression	5 (6.7)	10 (21.7)	<b>0.02</b>	<b>7.16</b> (1.39–36.90)	
Chronic kidney disease	4 (5.3)	4 (8.7)	0.06		
Cerebrovascular disease	2 (2.7)	4 (8.7)	0.19		
Chronic obstructive lung disease	2 (2.7)	3 (6.5)	0.36		
Signs and symptoms <sup>a</sup>					
Fever (Tc > 37.3 °C)	69 (92.0)	42 (91.3)	1		
Shortness of breath	41 (54.7)	22 (47.8)	0.57		
Dry cough	39 (52.0)	22 (47.8)	0.71		
Asthenia	20 (26.7)	7 (15.2)	0.17		
Diarrhea	4 (5.3)	6 (13.0)	0.17		
Nausea and vomiting	5 (6.7)	4 (8.7)	0.72		
Mental confusion	3 (4.0)	4 (8.7)	0.42		
Laboratory findings <sup>a</sup>					
Lymphopenia	66 (88.0)	41 (89.1)	0.88		
Hypoxemic respiratory failure	48 (63.1)	29 (63.0)	0.69		
AST > 40 UI/L	37 (48.6)	18 (39.1)	0.43		
ALT > 40 UI/L	29 (38.1)	15 (32.6)	0.56		
Thrombocytopenia	17 (22.3)	18 (39.1)	0.08		
Leukopenia	11 (14.4)	10 (21.7)	0.46		
Inflammatory markers <sup>a</sup>					
C-reactive protein > 5 mg/L	68 (89.4)	41 (89.1)	1		<b>0.022</b>
D-dimer > 500 µg/L	59 (78.6)	43 (93.4)	0.06		
IL-6 ≥ 35 ng/L	33 (43.4)	31 (67.3)	<b>0.02</b>	<b>3.70</b> (1.21–11.35)	



**Table 2** continued

Characteristics	Patients without PVS (N = 75)	Patients with PVS (N = 46)	<i>p</i>	OR (95% CI)	<i>p</i>
Radiologic findings <sup>a,b</sup>					
Interstitial pattern	39/72 (54.2)	18/45 (40.0)	0.18		
Bilateral consolidation	31/72 (43.1)	19/45 (42.2)	1		
Monolateral consolidation	18/72 (25.0)	13/45 (28.9)	0.67		
Absence of lesions	7/72 (9.7)	7/45 (15.6)	0.38		
Pleural effusion	1/72 (1.4)	2/45 (4.4)	0.55		
Time from onset of symptoms to diagnosis (median, IQR)	7.0 (4.0–10.0)	3.5 (2.0–7.0)	<b>0.001</b>	<b>0.77</b> (0.66–0.90)	<b>0.001</b>
Treatments					
Hydroxychloroquine	68 (90.7)	41 (89.7)	0.76		
Darunavir/ritonavir	47 (62.7)	25 (54.3)	0.44		
Corticosteroids	37 (49.3)	27 (58.7)	0.35		
Tocilizumab	21 (28.0)	12 (26.1)	1		
Oseltamivir	8 (10.7)	7 (15.2)	0.57		
Complications					
Non-invasive respiratory support	40 (53.3)	26 (56.6)	0.85		<b>0.006</b>
ICU admission	17 (22.7)	20 (43.5)	<b>0.02</b>		
Invasive mechanical ventilation	15 (20.0)	19 (41.3)	<b>0.01</b>	<b>4.95</b> (1.57–15.59)	
Septic shock	4 (5.3)	6 (13.0)	0.17		
Continuous renal replacement therapy	2 (2.7)	5 (10.9)	0.10		
All-cause in-hospital mortality	6 (8.0)	6 (13.6)	0.36		

*AKI* acute kidney injury, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ICU* intensive care unit, *IQR* interquartile range

Significant *p* values are shown in bold.

<sup>a</sup> These data were collected at the time of diagnosis of SARS-CoV-2 infection

<sup>b</sup> Chest CT was performed in 117 out of 121 patients at the time of SARS-CoV-2 infection

[25]. Moreover, since the duration of viral shedding is often used as an endpoint for clinical trials evaluating new antivirals [26], we suggest a stratification based on underlying comorbidities or IL-6 levels in order to avoid imbalances between treatment arms.

This study has some limitations that should be assessed. First, we excluded from our study

population those patients who early died or were discharged early. Accordingly, the percentage of patients with PVS may have been different if we could have obtained information about all patients consecutively hospitalized for SARS-CoV-2 infection at our hospital. Second, we did not analyze the viral load; therefore, quantification methods could impact our

conclusions. Third, because we did not perform daily collection of nasopharyngeal swabs we were not able to estimate the overall duration of viral shedding. Fourth, we only included hospitalized patients with severe SARS-CoV-2 infection; accordingly, our findings could not be extended to patients with mild or moderate disease who were not admitted to the hospital or discharged early.

## CONCLUSION

PVS is a common finding in hospitalized patients with SARS-CoV-2 infection, and is associated with immunosuppression and the need for mechanical ventilation. In our opinion, future studies should focus on the unknown meaning of this phenomenon in terms of infectivity and infection control measures.

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**Compliance with Ethics Guidelines.** The study was carried out in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of Liguria Region (N. CER Liguria 114/2020 - ID 10420). All patients provided verbal informed consent because of isolation precautions for performing nasopharyngeal swab according to the local protocol approved by hospital authorities, for data collection and for participation in the study.

**Data Availability.** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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