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Risk and predictive factors of prolonged viral RNA shedding in upper respiratory specimens in a large cohort of COVID-19 patients admitted to an Italian reference hospital

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ABSTRACT

Background: Limited data are available about the predictors and outcomes associated with prolonged SARS-CoV-2 RNA shedding (VS).

Methods: A retrospective study including COVID-19 patients admitted to an Italian hospital between March 1 and July 1, 2020. Predictors of viral clearance (VC) and prolonged VS from the upper respiratory tract were assessed by Poisson regression and logistic regression analyses. The causal relation between VS and clinical outcomes was evaluated through an inverse probability weighted Cox model.

Results: The study included 536 subjects. The median duration of VS from symptoms onset was 18 days. The estimated 30-day probability of VC was 70.2%. Patients with comorbidities, lymphopenia at hospital admission, or moderate/severe respiratory disease had a lower chance of VC. The development of moderate/severe respiratory failure, delayed hospital admission after symptoms onset, baseline comorbidities, or D-dimer >1000 ng/mL at admission independently predicted prolonged VS. The achievement of VC doubled the chance of clinical recovery and reduced the probability of death/mechanical ventilation.

Conclusions: Respiratory disease severity, comorbidities, delayed hospital admission and inflammatory markers negatively predicted VC, which resulted to be associated with better clinical outcomes. These findings highlight the importance of prompt hospitalization of symptomatic patients, especially where signs of severity or comorbidities are present.

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Introduction

The emergence and rapid spread of the COVID-19 outbreak, caused by SARS-CoV-2, has become a global health emergency and one of our century's greatest challenges. As of February 24, 2021, approximately 111 million confirmed cases and more than 2.4 million deaths had been reported worldwide (Anon, 2020).

Several reports have shown that the shedding profile of SARS-CoV-2 differs from other coronaviruses but is similar to that of the influenza virus (To et al., 2020; He et al., 2020). Viral RNA can be detected in the

upper respiratory tract (URT) a few days before symptoms onset, subsequently peaks within the first week of infection, and then gradually declines over time (To et al., 2020; He et al., 2020; Wolfel et al., 2020). According to recent evidence, the median duration of SARS-CoV-2 shedding in the URT is 17 days (Cevik et al., 2020). However, longer durations, up to 83 days, have been described (Li et al., 2020a). In several studies, mostly conducted in small cohorts, prolonged viral RNA shedding (VS) has been variously associated with male sex (Xu et al., 2020), older age (Wang et al., 2020; Du et al., 2020), disease severity (Xu et al., 2020; Du et al., 2020; Zhou et al.,

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2020a; Liu et al., 2020a; Zheng et al., 2020), delayed hospital admission/therapy start after symptoms onset (Xu et al., 2020; Qi et al., 2020; Fu et al., 2020) and comorbidities (Fu et al., 2020).

Several studies have questioned the correlation between the duration of VS and the duration of infectivity, showing that live virus can be cultured from respiratory samples for a significantly shorter time compared to detection of viral RNA by molecular methods, with a probability of detecting infectious virus lower than 5% after 15 days from symptoms onset (Wolfel et al., 2020; van Kampen et al., 2021). Although these findings have led to the update of isolation criteria in several international guidelines (WHO, 2020; CDC, 2020), decisions on infection prevention are still widely guided by strategies based on viral RNA detection. Therefore, further investigation on duration of VS and factors associated with prolonged shedding carried out on larger populations, may help to improve clinical management of COVID-19 patients.

Based on a large cohort of patients with COVID-19 admitted to an Italian reference hospital, this study aimed to estimate the time to SARS-CoV-2 clearance from the URT, identify predictive factors of both viral clearance (VC) and prolonged VS, and explore associations between VC and clinical outcomes.

Methods

Study design and population

We undertook an observational, retrospective, single-centre study including all consecutive adult patients with a confirmed diagnosis of SARS-CoV-2 infection, made by detection of viral RNA from a respiratory specimen, admitted to the National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS (Rome, Italy) between March 1 and July 1, 2020. Patients who did not have a date for symptoms onset or at least one post-diagnosis follow-up nasopharyngeal (NPS) or throat swab (TS) for SARS-CoV-2 were excluded from the analysis.

Data collection

Epidemiological, demographic, clinical, and laboratory data and information on treatments and outcomes of all patients with a confirmed COVID-19 diagnosis were collected and recorded using a standardized electronic database (ReCOVery study).

The study was approved by our local ethics committee.

Virological assessment and clinical management

For all included patients, the diagnosis of SARS-CoV-2 infection was confirmed on at least 1 respiratory specimen by the detection of SARS-CoV-2 RNA through real-time polymerase chain reaction (RT-PCR) targeting the E and RdRp viral genes (Corman et al., 2020). Respiratory tract specimens included were NPS, TS, sputum, bronchoaspirate (BAS) and bronchoalveolar lavage (BAL) fluid. During hospitalization, all patients underwent follow-up NPS and TS to assess VC. The frequency of NPS or TS follow-up was an average of every 3 days (interquartile range [IQR] 1–4) but it was not completely consistent as it depended on the treating physician's judgment and the hospital's internal protocol. The therapeutic management of patients was based on internal hospital protocol, national and international guidelines and clinical judgment, according to the best evidence available at the time.

Definitions

VC was defined as negative RT-PCR for SARS-CoV-2 on 2 consecutive NPS or TS. We considered acceptable a negative viral RNA on a single NPS or TS if it was the last available sample.

Prolonged VS was defined as detecting SARS-CoV-2 RNA on respiratory specimens for >18 days (the median duration of VS in our population). We considered the longest shedders as those patients in whom the detection of SARS-CoV-2 RNA on respiratory specimens was ≥ 26 days (75^o percentile of VS duration in our population).

We defined clinical recovery as being discharged from the hospital (including transfer to a non-acute healthcare facility) or weaning off oxygen in patients who underwent oxygen therapy during the hospitalization who had not needed it before admission.

The severity of respiratory disease was defined as mild, for a ratio of arterial oxygen partial pressure (PaO₂) to fractional inspired oxygen (FiO₂) between ≤ 300 mmHg and >200 mmHg; moderate, PaO₂/FiO₂ ratio between ≤ 200 mmHg and >100 mmHg; severe, PaO₂/FiO₂ ratio ≤ 100 mmHg (ARDS Definition Task Force, 2012).

Statistical analysis

Continuous and categorical variables were expressed as medians with IQRs and as numbers (%), respectively. The cumulative probability of achieving VC was estimated by Kaplan–Meier curves in the overall population and after stratifying by the severity of respiratory disease developed during hospitalization. In this latter analysis, the differences between groups were compared by the log-rank statistic test. Multivariable Poisson regression analysis was used to assess independent risk factors of VC, and multivariable logistic regression analysis to identify predictive factors of prolonged VS. In both multivariable analyses, significant factors identified on univariate analysis and other *a priori* identified confounders (age, sex) were included as covariates in the multivariable models. The causal relation between the duration of VS and the probability of positive (clinical recovery) and negative (invasive mechanical ventilation or death) clinical outcomes were evaluated using an inverse probability weighted (IPW) multivariable Cox model. The following factors were *a priori* included as covariates in the model: sex, age, number of comorbidities, antiviral therapy (yes vs no), immunomodulatory therapy and PaO₂/FiO₂ ratio during hospitalization. In all the performed analysis, the date of symptoms onset was considered as the baseline date. All statistical analyses were performed using STATA (version 15.1, College Station, Texas, USA). A *P*-value <0.05 indicated conventional statistical significance.

Results

Patients' characteristics at hospital admission, therapeutic aspects and main clinical outcomes

Overall, 630 adult subjects were admitted to our centre with a confirmed COVID-19 diagnosis during the observation period. After excluding 94 patients for whom the date of symptoms onset was not available (*n* = 89) or who did not have any follow-up swabs after diagnosis (*n* = 5), a total of 536 patients were included in the analysis. The main characteristics at hospital admission are shown in Table 1. The majority of subjects were male (64.6%), with a median age of 63 years (IQR 51–75). Most patients (63.8%) had at least one underlying concomitant disease, including diabetes (14%), hypertension (40.3%) and heart disease (24.6%). At hospital admission, which occurred within a median time of 8 days (IQR 4–11) from symptoms onset, 92% of patients had evidence of lung involvement, and approximately one third showed respiratory failure with a PaO₂/FiO₂ ratio of ≤ 300 mmHg. Antiviral therapy was started in 454 patients (84.7%), soon after hospital admission for most

Table 1
Patients' clinical characteristics on hospital admission, therapeutic aspects and main clinical outcomes.

Clinical characteristics at hospital admission	Overall Sample (N = 536)
Gender, n (%)	
Male	346 (64.6 %)
Age, years	
Median (IQR)	63 (51–75)
Comorbidities, n (%)	
None	194 (36.2 %)
1	133 (24.8 %)
2	78 (14.6 %)
≥3	131 (24.4 %)
Diabetes	75 (14.0 %)
Hypertension	216 (40.3 %)
Cardiovascular	132 (24.6 %)
Respiratory, including asthma	94 (17.5 %)
Neoplasm	54 (10.1 %)
Days from symptoms onset to hospital admission	
Median (IQR)	8 (4–11)
Severe respiratory disease, n (%)	
PaO ₂ /FiO ₂ ≤ 300 mmHg	175 (32.7 %)
PaO ₂ /FiO ₂ ≤ 200 mmHg	70 (13.1 %)
Radiologic evidence of pneumonia, n (%)	
yes	493 (92.0 %)
Inflammatory index, n (%)	
Lymphocytes count < 1.0 × 10 ⁹ /L	207 (38.6 %)
D-dimer > 10 ³ ng/mL	304 (56.7 %)
Serum Ferritin > 500 ng/mL	206 (38.4 %)
Treatment and main outcomes during hospitalization	
Days from symptoms onset to antiviral therapy	
Median (IQR)	8 (5–11)
Antiviral treatment, n (%)	
None	82 (15.3 %)
Yes	454 (84.7 %)
- PI/b	115 (25.3 %)
- HCQ	83 (18.3 %)
- PI/b + HCQ	226 (49.7 %)
- Remdesivir	30 (6.6 %)
✓ Remdesivir alone	5 (16.7 %)
✓ After PI/b	7 (23.3 %)
✓ After HCQ	6 (20.0 %)
✓ After PI/b + HCQ	12 (40.0 %)
Immunomodulatory therapy, n (%)	
Cytokines inhibitors	106 (19.8 %)
Corticosteroids	224 (41.8 %)
- Not associated with antivirals	14 (6.3 %)
- Associated with antivirals	210 (93.7 %)
Duration of viral shedding from symptoms onset,	
Median (IQR)	18 (12–26)
Patients achieving viral clearance, n (%)	
Total	359 (66.9 %)
Patients who developed respiratory failure during hospitalization, n (%)	
PaO ₂ /FiO ₂ ≤ 300 mmHg	128 (23.8 %)
PaO ₂ /FiO ₂ ≤ 200 mmHg	75 (14.0 %)
Patients who needed respiratory support, n (%)	
Oxygen therapy	403 (75.3 %)
NPPV	133 (24.8 %)
IMV	51 (9.5 %)
Hospitalization outcomes, n (%)	
Death	63 (11.8 %)
Discharged at home	372 (69.4 %)
Transferred to non-acute healthcare facility	101 (18.8 %)
Death with positive NPS/TS	46 (8.6 %)
Discharged/transferred with positive NPS/TS	122 (24.4 %)
Hospitalization length, days	
Median (IQR)	12 (7–20)
Follow-up, days	
Median (IQR)	18 (12–26)

Abbreviations: n, number; IQR, interquartile range; PaO₂, arterial oxygen partial pressure; FiO₂, fractional inspired oxygen; HCQ, hydroxychloroquine; PI/b, boosted protease inhibitors; NPPV, Non-Invasive Positive Pressure Ventilation; IMV, Invasive Mechanical Ventilation, NPS, nasopharyngeal swab; TS, throat swab.

patients, with protease inhibitors in 25.3%, hydroxychloroquine in 18.3%, and protease inhibitors combined with hydroxychloroquine in 49.7%. A minority of patients (6.6%) were enrolled in randomized clinical trials and started remdesivir, most of them (83.3%) after initial antiviral therapy. Immunomodulatory therapy with cytokines inhibitors was given to 19.8% of patients and systemic corticosteroids to 41.8%, mostly (93.7%) in association with antivirals (specifically, in 61 patients with protease inhibitors, 22 with hydroxychloroquine, 107 with protease inhibitors plus hydroxychloroquine, and 20 with remdesivir).

The median time of VC from URT was 18 days (IQR 12–26) from symptoms onset. Over a median time of hospitalization of 12 days (IQR 7–20), 473 patients (88.2%) were discharged or transferred to non-acute healthcare facilities, and 63 (11.8%) died. During hospitalization, 128 patients (23.8%) developed respiratory failure, which was moderate to severe (PaO₂/FiO₂ ratio ≤200 mmHg) in 75/128 patients. Supplemental oxygen was given to 403 patients (75.3%), including non-invasive positive pressure ventilation in 133 and invasive mechanical ventilation in 51 (Table 1). The majority of the longest shedders (≥26 days from symptoms onset) were male (65.4%) with a median age of 68 years (IQR 53–78), and 78.4% of them had at least 1 underlying concomitant disease. For the longest shedders, hospital admission occurred within a median of 12 days (7–24) and 37.4% of them had a PaO₂/FiO₂ ratio of ≤300 mmHg at admission.

Probability of viral clearance and associated risk factors

Over a median follow-up of 18 days (IQR 12–26), 359 patients (66.9%) achieved VC. In 34 (9.5%) of these 359 patients the achievement of VC was considered after a single negative swab since it was the last available sample. Of note, four of these 34 patients died after the last negative follow-up specimen, while 8 had intermittent VS before the last available swab. The shortest duration of VS observed in our population was 1 day, while the longest was 73 days. In the overall population, the estimated probability of achieving VC at 20 days and 30 days from symptoms onset was 44.5% (95% CI: 40–49) and 70.2% (95% CI: 65–75), respectively (Figure 1a). After stratifying by the severity of respiratory disease, the cumulative probability of VC from the URT was significantly lower in patients who developed moderate/severe respiratory failure (PaO₂/FiO₂ ratio ≤200 mmHg) compared to those who maintained a PaO₂/FiO₂ ratio of >200 mmHg during hospitalization (P = 0.002, Figure 1c). The probability of VC did not differ after stratifying for less severe respiratory failure (Figure 1b).

At the multivariable Poisson regression analysis, patients who developed moderate/severe respiratory failure (PaO₂/FiO₂ ≤ 200 mmHg) either at hospital admission or during hospitalization were less likely to achieve VC (adjusted incidence rate ratio [aIRR] 0.42, 95% CI: 0.27–0.66, P < 0.001). Similarly, patients who had underlying comorbidities (aIRR 0.88 for each additional comorbidity, 95% CI: 0.80–0.96, P = 0.004) and lymphopenia (lymphocytes count below 1.0 × 10⁹/L) at hospital admission (aIRR 0.75, 95% CI: 0.58–0.98, P = 0.032) were found to be associated with a lower probability of VC (Table 2).

Risk factors for prolonged viral shedding

Overall, 258 patients showed detectable SARS-CoV-2 RNA in URT specimens for more than 18 days. Analysing predictive factors of prolonged VS by multivariable logistic regression analysis, having underlying comorbidities (adjusted odds ratio [aOR] 1.25 for each additional comorbidity, 95% CI: 1.04–1.51, P = 0.019) and

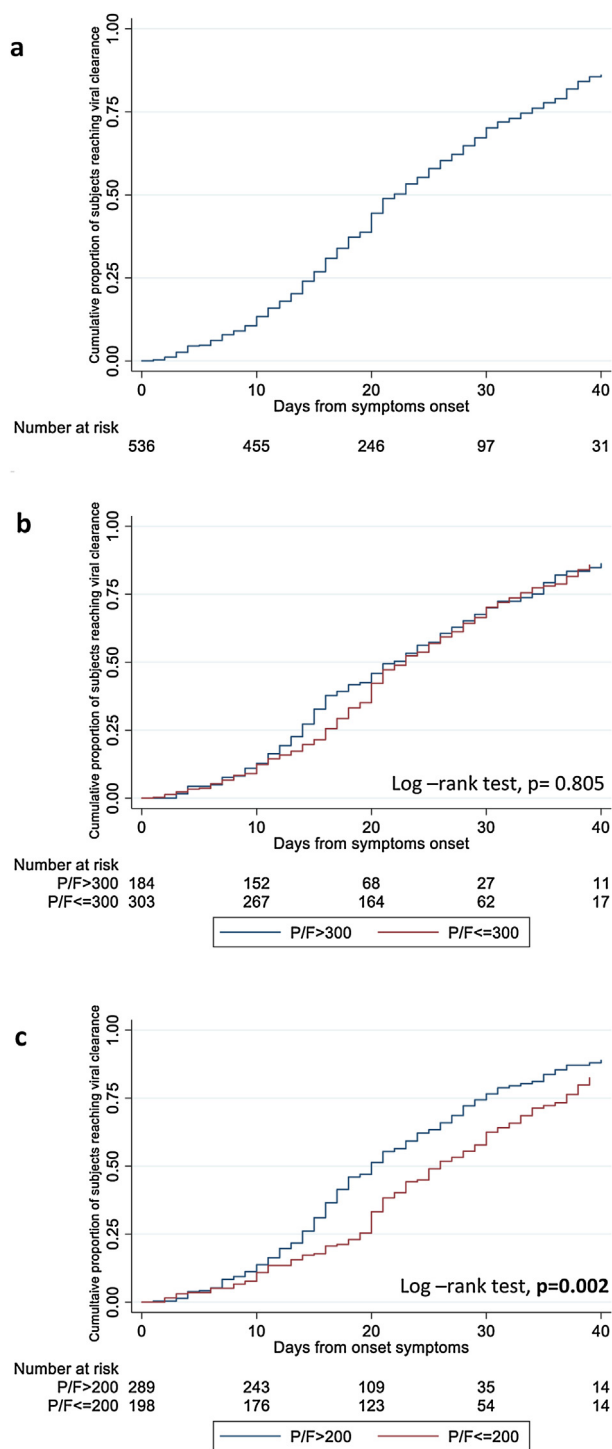


Figure 1. Kaplan-Meier curves estimating the cumulative probability of viral clearance in total population (a) and after stratifying by the severity of respiratory disease [PaO₂/FiO₂ ratio ≤300 mmHg versus >300 mmHg (b) and PaO₂/FiO₂ ratio ≤200 mmHg versus >200 mmHg (c)].

having developed moderate/severe respiratory failure (PaO₂/FiO₂ ratio ≤200 mmHg) during hospitalization (aOR 2.65, 95% CI: 1.39–5.05, P=0.003) were significantly associated to increased odds of slower VC. Additionally, delayed hospital admission after illness onset (aOR 1.18 for each additional day of symptoms before hospitalization, 95% CI: 1.12–1.23, P<0.001) and a high value of D-dimer (above 1000 ng/mL) at admission (aOR 1.76, 95% CI: 1.04–2.99, P=0.035) significantly predicted a prolonged VS (Table 3).

Finally, the use of steroid therapy showed a trend toward significance as a predictive factor of prolonged VS (aOR 1.79, 95% CI: 0.95–3.39, P=0.073).

Association between viral RNA shedding and clinical outcomes

The potential causal relationship between the achievement of VC and the occurrence of the main clinical outcomes was explored by a multivariable IPW Cox model (Figure 2). After controlling for the main confounding factors listed in Methods, we found that VC strongly predicted clinical recovery (adjusted hazard ratio [aHR] 2.17, 95% CI: 1.56–3.00, P<0.001) and reduced the probability of invasive mechanical ventilation or death by more than 60% (aHR 0.36, 95% CI: 0.18–0.69, P=0.002).

Discussion

This retrospective study focused on the duration of VS from the URT and the factors associated with both VC and prolonged VS.

In our cohort the median duration of VS, from symptoms onset to VC, was 18 days. This finding is in line with a recent systematic review and metaanalysis, which reported a mean duration of viral detection from the URT of 17 days, from an analysis of 43 studies including 3229 subjects (Cevik et al., 2020). However, the median duration of SARS-CoV-2 shedding in the respiratory tract varies widely in the different studies, ranging between 11 and 31 days (Li et al., 2020a; Xu et al., 2020; Zhou et al., 2020a; Qi et al., 2020; Zhou et al., 2020b). This variability could be partially explained by the heterogeneity of study populations, particularly regarding the clinical conditions, and the different kind of specimens considered. Similarly, also the maximum persistence of SARS-CoV-2 RNA differ significantly among the studies, with the longest described VS duration of 83 days, lasting even after symptoms resolution and seroconversion (Li et al., 2020a). Our study found a maximum VS duration of 73 days, one of the longest reported to date. In our cohort, patients with the longest VS duration were mainly characterized by advanced age, underlying comorbidities and a delayed hospital admission (>10 days after illness onset) and a severe clinical presentation of the disease.

In the current study, the severity of illness, delayed admission to hospital after symptoms onset, presence of underlying comorbidities and alteration of inflammatory index at hospital admission were identified as independent risk factors of delayed VC and/or prolonged VS.

The association between the severity of COVID-19 disease and duration of VS has already been widely reported (Xu et al., 2020; Du et al., 2020; Zhou et al., 2020a; Liu et al., 2020a; Zheng et al., 2020). Although several studies have described similar (To et al., 2020; Cevik et al., 2020; Yilmaz et al., 2021) or even longer (Cevik et al., 2020) duration of VS in the URT in mild compared to severe cases, the majority of reports found that patients with more severe illness shed viral RNA for a longer period (Cevik et al., 2020; Xu et al., 2020; Du et al., 2020; Zhou et al., 2020a; Liu et al., 2020a; Zheng et al., 2020; Fontana et al., 2020). However, the definition of severity among these studies is not homogeneous. Most studies evaluated the differences in VS duration between mild and severe cases (Du et al., 2020; Liu et al., 2020a; Zheng et al., 2020). Conversely, Zhou et al. compared severe versus critical cases (Zhou et al., 2020), and in Xu et al., invasive mechanical ventilation was used as a proxy for illness severity (Xu et al., 2020). In this study, we used PaO₂/FiO₂ ratio to define disease severity, according to the Berlin definition of acute respiratory distress syndrome (ARDS Definition Task Force, 2012). We found that a PaO₂/FiO₂ ratio of ≤200, corresponding to moderate to severe respiratory failure (ARDS Definition Task Force, 2012), was associated with a delayed VC/prolonged VS. Of note, in a recent study, the correlation

Table 2
Predictive factors of viral clearance by Poisson regression analysis.

	Crude IRR (95 % CI)	p-value	Adjusted IRR (95 % CI)	p-value
Gender				
Male vs Female	1.01 (0.82–1.26)	0.904	1.03 (0.82–1.29)	0.798
Age				
For 10 years older	0.96 (0.90–1.02)	0.217	1.03 (0.95–1.12)	0.419
Underlying comorbidities				
For each more	0.90 (0.84–0.97)	0.005	0.88 (0.80–0.96)	0.004
Inflammatory index at hospital admission				
Lymphocytes count < $1.0 \times 10^9/L$	0.69 (0.54–0.87)	0.002	0.75 (0.58–0.98)	0.032
D-dimer > 10^3 ng/mL	1.16 (0.89–1.51)	0.271		
Serum Ferritin > 500 ng/mL	1.07 (0.82–1.39)	0.631		
Severity of respiratory disease ^a				
PaO ₂ /FiO ₂ ≤ 200 versus > 200 mmHg	0.61 (0.47–0.79)	<0.001	0.42 (0.27–0.66)	<0.001
Time from symptoms onset to admission				
for 1 day more	0.98 (0.97–0.99)	<0.001	1.00 (0.99–1.00)	0.378
Antiviral therapy ^a				
Yes versus no	2.81 (2.23–3.53)	<0.001	1.16 (0.77–1.74)	0.490
Corticosteroids ^a				
Yes versus no	1.57 (1.25–1.95)	<0.001	1.12 (0.85–1.46)	0.419
Cytokines inhibitors				
Yes versus no	1.04 (0.81–1.33)	0.783	0.95 (0.73–1.23)	0.677

Abbreviations: IRR, incidence rate ratio; CI, confidence interval.

^a Time updated variable.**Table 3**
Predictive factors of prolonged viral shedding (> 18 days) by Logistic regression analysis (on 438 patients^a).

	Crude OR (95 % CI)	p-value	Adjusted OR (95 % CI)	p-value
Gender				
Female vs Male	0.72 (0.48–1.08)	0.112	0.90 (0.55–1.46)	0.668
Age				
For 10 years older	1.15 (1.02–1.30)	0.022	1.02 (0.85–1.23)	0.804
Underlying comorbidities				
For each more	1.22 (1.07–1.39)	0.003	1.25 (1.04–1.51)	0.019
Inflammatory index at hospital admission				
Lymphocytes count < $1.0 \times 10^9/L$	1.05 (0.77–1.42)	0.764		
D-dimer > 10^3 ng/mL	0.90 (0.84–0.96)	0.002	1.76 (1.04–2.99)	0.035
Serum Ferritin > 500 ng/mL	0.96 (0.90–1.02)	0.153		
Severity of respiratory disease at hospital admission,				
PaO ₂ /FiO ₂ ≤ 200 versus > 200 mmHg	2.32 (1.23–4.39)	0.010	0.86 (0.38–1.95)	0.711
Severity of respiratory disease during hospitalization,				
PaO ₂ /FiO ₂ ≤ 200 versus > 200 mmHg	3.05 (1.98–4.69)	<0.001	2.65 (1.39–5.05)	0.003
Time from symptoms onset to admission				
for 1 day more	1.13 (1.09–1.18)	<0.001	1.18 (1.12–1.23)	<0.001
Antiviral therapy				
Yes versus no	1.24 (0.74–2.09)	0.413	1.75 (0.81–3.81)	0.158
Corticosteroids				
Yes versus no	2.75 (1.64–4.59)	<0.001	1.79 (0.95–3.39)	0.073
Cytokines inhibitors				
Yes versus no	2.77 (1.86–4.13)	<0.001	1.53 (0.90–2.59)	0.116

Abbreviations: OR, odds ratio; CI, confidence interval.

^a Excluding patients who died before 18 days without virological clearance and those discharged with viral shedding before 18 days in whom the data about the follow-up swabs performed after hospitalization were not available.

between disease severity and longer persistence of SARS-CoV-2 RNA in respiratory tract samples has also been confirmed using virus cultures, which are considered a more reliable index of infectivity compared to molecular methods (van Kampen et al., 2021). The association between illness severity and prolonged VS, which has previously been described for MERS and influenza virus (Oh et al., 2016; Wang et al., 2018), might be partially explained by the higher viral loads reported in severe COVID-19 cases compared to mild ones (He et al., 2020; Liu et al., 2020a; Zheng et al., 2020).

Noteworthy, in the current study, the relationship between VS duration and the clinical course of COVID-19 appeared to be bidirectional. Consistent with data from other coronavirus and influenza virus infections (Wang et al., 2018; Arabi et al., 2017), we found that VC from the URT almost doubled the odds of clinical recovery and reduced the risk of worse clinical outcomes. The ongoing pro-inflammatory stimulus due to the persistence of

SARS-CoV-2 in the respiratory tract and the resulting organ damage may contribute to explaining this finding.

Although comorbidities have been identified as one of the main prognostic factors for COVID-19 severity, only a few studies have reported an association with the duration of VS (Fu et al., 2020; Xiao et al., 2020). Several conditions, including coronary heart disease (Fu et al., 2020), hypertension (Xiao et al., 2020) and diabetes (Xiao et al., 2020), have been linked to a longer persistence of SARS-CoV-2 RNA in respiratory tract. Our study did not find an association between specific comorbidities and the persistence of viral RNA. Rather we observed that patients with underlying comorbidities were more likely to have both slower VC and prolonged viral detection with an increased risk for each additional comorbidity.

In our cohort, we found that deferred hospital admission after the onset of symptoms increased the probability of prolonged VS

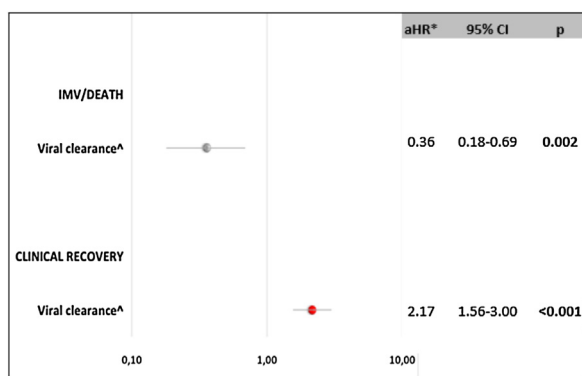


Figure 2. Causal relation between viral clearance and main clinical outcomes by Inverse Probability Weighted Cox regression Analysis.

Abbreviations: aHRadjusted hazard ratio; CIconfidence interval; IMVinvasive mechanical ventilation

^ΔAfter adjusting for gender, age, number of comorbidities, use of antiviral therapy (yes versus no), use of cytokines inhibitors (yes versus no), use of steroids (yes versus no), PaO₂/FiO₂ ratio (≤ 200 mmHg versus > 200) during hospitalization (time updated variable).

by approximately 15% per day. This finding, already reported in previous studies (Xu et al., 2020; Qi et al., 2020; Li et al., 2020b; Xudan et al., 2020), may suggest a potential effect of antiviral treatments on SARS-CoV-2 clearance, as demonstrated for influenza A infection (Wang et al., 2018). This hypothesis is also supported by the viral dynamics of SARS-CoV-2 characterized by a peak in viral load at the time of, or shortly before, symptoms onset. However, contrary to influenza A infection, none of the antiviral regimens considered in this study have shown clear advantages in limiting the duration of viral shedding (Cevik et al., 2020). Additionally, in our cohort, the use of antiviral therapy was not associated with either the probability of VC or prolonged VS. An alternative explanation for this result is that the application of general supportive measures alone might have reduced the length of VS. The association between the time from symptoms onset to hospitalization and the duration of SARS-CoV-2 shedding is a key finding of this study which highlights the importance of a medical strategy based on rapid testing of symptomatic patients and prompt hospitalization and management of the infected patients.

The role of corticosteroids in the management of COVID-19 is still a matter of debate. Although data from recent randomised clinical trials have shown a potential beneficial effect of steroid therapy on survival in severe/critically-ill patients (The WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group, 2020; RECOVERY Collaborative Group et al., 2020), their impact on VS remains more uncertain, with some evidence indicating a possible association with a delayed VC (van Paassen et al., 2020). In our cohort, we did not observe a clear association between the use of corticosteroids and a prolonged VS, as previously described in both SARS-CoV-2 and other coronavirus infections (Li et al., 2020b; Arabi et al., 2018). However, further evidence from ongoing clinical trials might better clarify the role of corticosteroids in COVID-19 treatment.

Finally, alteration of pro-inflammatory markers, and particularly lymphopenia and elevated D-dimer, at the time of hospital admission was found to be an independent risk factor of prolonged VS. Given the role of the immune response in the clinical course of COVID-19, these indices have already been identified as markers of disease severity (Zhou et al., 2020a), and a recent study found that the alteration of the inflammatory markers was more frequent in patients with prolonged VS (Qi et al., 2020; Liu et al., 2020b). However, an independent association with VS has not previously been observed, and further studies are warranted to confirm this result.

Our study has several limitations. First, the study could be prone to bias related to unmeasured confounders due to its observational nature. Second, the retrospective collection of medical records may have introduced bias due to potentially inaccurate data reporting. Third, the estimated duration of VS could have been influenced by heterogeneity in the frequency of specimen collection and the type of respiratory specimen used. Additionally, the lack of any quantitative determination of viral load, even through an indirect measure such as cycle-threshold value, do not allow us to draw any conclusion about the potential infectiousness of long-term shedders. Finally, the single-centre nature of the study may limit the generalizability of our findings. However, the study's main strength is its large sample size; to our knowledge, this is one of the largest cohorts in which the duration of SARS-CoV-2 RNA shedding has been investigated. Thus, this study provides significant information on the dynamics of VS and the determinants of delayed VC.

In conclusion, through a large retrospective cohort study, we found that severity of respiratory disease, delayed admission to hospital after the onset of symptoms, presence of underlying comorbidities and alteration of pro-inflammatory markers at the time of hospitalization independently predicted a longer persistence of SARS-CoV-RNA in the upper respiratory tract. Furthermore, our data showed that a more rapid achievement of VC was independently associated with better clinical outcomes and survival. These findings suggest a more rapid VC and limit on the worse clinical outcomes could be achieved through a medical strategy based on rapid testing of symptomatic subjects and prompt hospitalization and therapy initiation for patients with a confirmed diagnosis, especially where signs of severity and concomitant medical conditions are present. Infection control and prevention criteria might also consider the disease's clinical course, addressing test-based isolation strategies or longer isolation periods for patients with severe/critical COVID-19.

Author contributions

AM was a major contributor in writing the manuscript; PL analysed data; CC, RG, EL, AC, MBV, FT, SC, LL, FDG, GDO, FP, EN, CA, NP, GI, FV, EG, MRC have participated in the research and acquisition of data; AA made substantial contributions to the conception of the work and interpretation of data.

All authors read and approved the final manuscript.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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