Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Insight into the reason of prolonged viral RNA shedding in patients with COVID-19 infection

Dear Editor,

Severe acute respiratory syndrome coronavirus (SARS-CoV-2), a novel RNA coronavirus from the same family as SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), was identified in early January 2020 as the cause of a pneumonia epidemic affecting the city of Wuhan, the capital of Hubei province, from where it rapidly spread across China.[1,2] From China, infection rapidly reached Europe and USA, with the number of new cases currently increasing every day.[3,4] The World Health Organization declared the coronavirus diseases 2019 (COVID-19) as a pandemic due to the widespread infectivity and high contagion rate.[5] According to the WHO's guidelines on clinical management, a patient can be discharged from hospital after two consecutive negative real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) tests at nasopharyngeal swabs at least 24 h apart in a clinically recovered patient (https://ecdc.europa.eu/en/publications-data/covid-19-guidance-discharge-and-ending-isolation). The pattern of SARS-CoV-2 RNA shedding during the course of COVID-19 infection has not been well characterized to date. Several reports assessed the possible recurrence of COVID-19 infection,[6,7] but little information is known about the prolonged persistence of viral RNA shedding; in particular, two recent, small, retrospective studies found that male sex, delayed admission to hospital after illness onset and disease severity were all associated with a prolonged SARS-CoV-2 RNA shedding.[8,9] Recently, a paper by Fang et al.[10] compared the time of viral shedding of SARS-CoV-2 in patients hospitalized in intensive care unit (ICU) or not; in particular, they found that the time of viral shedding of blood, saliva and nasal samples was longer in ICU-patients compared to others, and exceeded two weeks in all the patients included. The authors suggest that these differences might be correlated with viral load, severity and invasive operations related to ICU hospitalization.

In the present paper we evaluated the factors associated with a prolonged SARS-CoV-2 RNA shedding in a large population of patients followed-up at ASST Rhodense from March to September 2020.

In this analysis, we included 681 subjects with a confirmed COVID-19 infection; patients were diagnosed by a nasopharyngeal swab between March 2010 and September 2010, and had a follow-up of at least 8 weeks. Population was divided into three groups according to the time needed to swab negativization (defined as two consecutive negative nasopharyngeal swabs repeated after 24–48 h from each other). Group A included 284 patients who got negative $<$ 3 weeks ("early" negativization); Group B included 225 patients who got negative after 3–6 weeks ("medium" negativization); Group C included 172 patients who got negative $>$ 6 weeks ("late" negativization). Clinical and demographical features were collected in the entire population, as were data on disease severity (need of hospitalization or not) and death. Continuous variables were expressed as mean values and standard deviations (SD) or as median values and interquartile ranges (IQR) according to variable skewness. Non-continuous variables were described as percentages. Normal distribution was tested using the Kolmogorov–Smirnov test and statistical parametric techniques were applied. The in-between groups comparison was performed by one-way analysis of variance ANOVA and p*ost-hoc* analyses using Scheffè test were performed when the main effect was significant. All data analysis was performed using the *MedCalc software* © (Ostend, Belgium, version 12.7.0) and significance was conventionally set at 5% ($p < 0.05$).

First of all, we assessed normal distribution of our sample and also identified little number of possible outliers Then, we preliminary divided total number of study population in 3 groups according to time to negativization. Table 1 summarized clinical and demographic characteristics of the population studied, according to the 3 groups considered. At univariate analysis, male gender was associated with a prolonged time to recovery ($p < 0.05$). Patients who recovered earlier were younger than others (mean age: Group "early" negativization 50.5 years, Group "medium" negativization 57 years, Group "late" negativization 65.8 years, $p < 0.05$). The percentage of hospitalized patients was higher, though not statistically significant among Group "late" negativization (75.6%) compared to others (Group "early" 41.4%, Group "medium" 48.4%, $p = 0.08$), as was the percentage of deaths (8.1% vs 5.3% Group "medium2 and 1.1% Group "early", $p = 0.09$). Subsequently, a stepwise multiple regression (backward model) was used to analyze main underlying relationship between study's parameters; with this specific analysis, we found only one strong relationship between time to negativization and age. Fig. 1 shows the correlation between older age and time to negativization evaluated by ANOVA statistical analysis.

In this paper we evaluated the factors associated with a prolonged viral shedding in subjects with COVID-19 infection. Current data on this aspect of the disease are lacking and mainly come from Chinese studies that usually include younger patients compared to European studies; moreover, current studies included a smaller population with a limited time of follow-up. In particular, the study by Xu et al.[8] defined as patients with a prolonged viral shedding those with a time to negativization $>$ 15 days; this issue has been overcome by the observation that most patients included in European cohorts recovered slower.[11] In this paper, at multivariate analysis male sex, delayed admission to hospital after symptoms onset and invasive mechanical ventilation were all factors statistically associated with a prolonged viral shedding; older age was associated only at univariate analysis. Similarly, in the study by Shi et al.[9] male gender, disease severity and lymphocyte count were all predictors of prolonged viral shedding. However, in

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Table 1
Demographic and clinical characteristics of the population according to the time to negativization. Group "early": ⟨ 3 weeks; Group "medium": 3–6 weeks; Group "late": ⟩ 6 weeks.

	Group "Early" ($n = 284$)	Group "Medium" ($n = 225$)	Group "Late" ($n = 172$)	p
Age, mean (95% CI)	50.5 (22–79)	57.0 (27–86)	65.8 (32–90)	< 0.05
Male, n (%)	208 (53.1)	105 (46.7)	75 (43.6)	< 0.05
Days to recovery, median (95% CI)	17 (9–21)	29 (22–41)	53 (43–111)	< 0.01
Hospitalization, n (%)	118 (41.5)	109 (48.4)	139 (75.6)	0.08
Deaths, n (%)	3 (1.1)	12 (5.3)	14 (8.1)	0.09

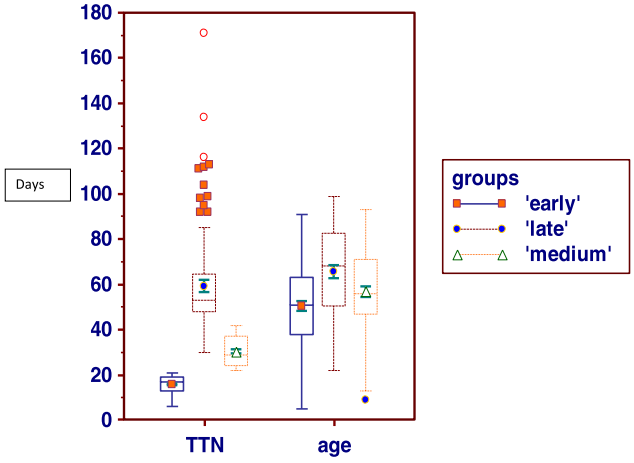

Fig. 1. Anova analysis on the correlation between age and time to negativization (TTN).

this study only 99 patients were included and the median follow-up was 28 days; furthermore, the median time of persistent viral shedding was 16 days and only 12 patients had detectable virus up to 30 days after symptoms onset. Also the study by Fang et al.[10] included a limited number of patients (32 patients) that were significantly younger than those included in the present paper. Furthermore, the possible correlation between the prolonged viral shedding and the severity of disease has been observed also in our univariate analysis. The differences among the baseline characteristics of our population and the patients included in the studies by Xu,[8] by Shi[9] and by Fang[10] can explain the different results obtained. In our study, though male sex was significantly associated with a prolonged viral shedding at univariate analysis, this finding was non confirmed at the stepwise multiple regression analysis; at this analysis, only age was associated with a prolonged viral shedding.

At our knowledge, this is the largest cohort study that evaluated the predictive factors of prolonged viral shedding in patients with COVID-19 infection. In this population, older age showed to be the strongest predictors of prolonged viral shedding. This finding may have a significant impact in the future management of the pandemic, especially in the elderly population.

Declaration of Competing Interest

No found. All the authors of the manuscript declare that they do not have any conflict of interest in connection with this paper.

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