PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Temporal profile and determinants of viral shedding and of viral clearance confirmation on nasopharyngeal swabs from SARS-CoV-2-positive subjects: a population-based prospective cohort study in Reggio Emilia, Italy.
AUTHORS	Mancuso, Pamela; Venturelli, Francesco; Vicentini, Massimo; Perilli, Cinzia; Larosa, Elisabetta; Bisaccia, Eufemia; Bedeschi, Emanuela; Zerbini, Alessandro; Giorgi Rossi, Paolo

VERSION 1 – REVIEW

REVIEWER	Lixin Xie College of Pulmonary and Critical Care Medicine Chinese PLA General Hospital Beijing China
REVIEW RETURNED	26-May-2020

GENERAL COMMENTS	1. In this manuscript, the authors reported that the diagnosis of SARS-CoV-2 infection was carried out on samples from nasopharyngeal swabs, we want to know the ways of nasopharyngeal swabs, that of the standard of nasopharyngeal swabs procedure directly determine the accuracy of SARS-CoV-2 nucleoid detection results. 2. It is known that SARS-CoV-2 nucleoid detection exists false negative, how to avoid? 3. Table 1: Disease severity classification: In table 1, the authors classified the enrolled patients as No access to Emergency Department or hospital, Emergency Department use only, and Hospitalization groups for analyzing the severity of COVID-19, to our knowledge, although some patients diagnosed not access to Emergency Department or hospital, it does not means that these patients are absolutely mild or asymptomatic, maybe some patients who getting worse go to other hospitals or fall in death at home. We suggest that it is better to classify the severity of COVIDS-19 in accordance with the following data, SOFA, APACH II, P/F ratio, pulmonary infiltration, lymphocyte counts. 4. In the manuscript, the authors reported that there were about one fifth (21.3%) of viral clearances in the follow-up period which were not confirmed by the second swab, suggesting that there was a high rate of false negatives in this population, the authors should discuss the reasons of false negatives in the revised manuscript.

REVIEWER	Aijun Pan the first affiliated hospital of USTC, China
REVIEW RETURNED	27-May-2020

GENERAL COMMENTS	In this manuscript entitled " Temporal profile and determinants of viral shedding and of viral clearance confirmation on nasopharyngeal swabs from SARS-CoV-2-positive subjects: a population-based study in Reggio Emilia ", the authors determined the timing of viral clearance (first negative RT-PCR on nasopharyngeal swab) and the probability of viral clearance confirmation (two consecutive negative swabs) in COVID-19 patients. This prospective cohort study, recruiting 1162 patients, describes the time characteristics of virus clearance and found one in five false negative tests. This study also revealed that the time to viral clearance increased with age and disease severity. The main limitation of this current study was that a few of covariates were included in multivariate logistic regression model, while some important indicators, such as previous complications, laboratory tests, and treatment, were not collected. Therefore, the risk factors derived from this model may not be comprehensive. Overall, the manuscript is concisely organized and written well. The following comments should be considered. 1. The authors described that “Death occurs in about 20% of cases…”. However, data from ECDC (reference 5) as of 25 March showed that Among hospitalized cases, severe illness was reported in 15% of cases, and death occurred in 12% of these cases. Which area does the high mortality rate of 20% refer to? 2. Data analysis showed that the time to viral clearance increased with age and disease severity. However, these two indicators are closely related according to previous studies. Multivariable regression showed increasing odds of in-hospital death associated with older age (odds ratio 1·10, 95% CI 1·03-1·17, per year increase; p=0·0043) in Zhou F’s study. (Lancet. 2020;395(10229):1054-1062.)

VERSION 1 – AUTHOR RESPONSE

Reviewers' Comments to Author:

Reviewer: 1
Reviewer Name: Lixin Xie
Institution and Country: College of Pulmonary and Critical Care Medicine, Chinese PLA General Hospital, Beijing, China
Please state any competing interests or state ‘None declared’: None declared

1. In this manuscript, the authors reported that the diagnosis of SARS-CoV-2 infection was carried out on samples from nasopharyngeal swabs, we want to know the ways of nasopharyngeal swabs, that of the standard of nasopharyngeal swabs procedure directly determine the accuracy of SARS-CoV-2 nucleoid detection results.
RE: We thank the reviewer for the comment. We have added methodological details on molecular diagnosis in the Methods. (see page 8, RT-PCR methodology section)

2. It is known that SARS-CoV-2 nucleoid detection exists false negative, how to avoid?
RE: We are all aware that a non-negligible proportion of SARS-CoV-2 RT-PCR tests provide false negative results. Indeed, one of the aims of our study was to assess the proportion of false negatives. Further, we reported and discussed the association between false negative results and time from disease onset and patient characteristics. We do not rule out the possibility that two consecutive

negative tests could be false negative tests, and we acknowledge this as a limitation of our study. Nevertheless, we use the occurrence of a positive test after a negative test in this context as a proxy of false negative. We describe the distribution of these unconfirmed negative tests and we try to identify the conditions under which they occur more frequently. Our findings provide evidence that may help to reduce false negative rates in routine surveillance of COVID-19 patients.(see page 12, Strengths and weaknesses of the study)

3. Table 1: Disease severity classification: In table 1, the authors classified the enrolled patients as No access to Emergency Department or hospital, Emergency Department use only, and Hospitalization groups for analyzing the severity of COVID-19, to our knowledge, although some patients diagnosed not access to Emergency Department or hospital, it does not means that these patients are absolutely mild or asymptomatic, maybe some patients who getting worse go to other hospitals or fall in death at home. We suggest that it is better to classify the severity of COVIDS-19 in accordance with the following data, SOFA, APACH II, P/F ratio, pulmonary infiltration, lymphocyte counts.
RE: We agree with the reviewer that the classification of disease severity in hospitalized, only ED visit and no hospital access could be limited. However, the population-based design is the main strength of our study and for non-hospitalised patients, clinical information are not available. We included a cohort of people living in the province of Reggio Emilia (northeastern Italy), whose six hospitals and primary care services were never overwhelmed during the worst period of the COVID-19 epidemic. Thus, we do not expect that our health care services were not able to provide patients with the care they needed. We have included a comment in the Methods section as well as the number and age of deaths in hospitalised and non-hospitalised patients to provide more information on the fatality rate in these three groups. Thank you for your comment (see page 9, Covariates; see pages 10-11 Results; see pages 12-13 Strengths and weaknesses of the study).

4. In the manuscript, the authors reported that there were about one fifth (21.3%) of viral clearances in the follow-up period which were not confirmed by the second swab, suggesting that there was a high rate of false negatives in this population, the authors should discuss the reasons of false negatives in the revised manuscript.
RE: We thank the reviewer for this comment. We have added to the Discussion two paragraphs on this point. (see page 14, Comparison with other studies and interpretation)

Reviewer: 2
Reviewer Name: Aijun Pan
Institution and Country: the first affiliated hospital of USTC, China
Please state any competing interests or state ‘None declared’: None declared

In this manuscript entitled " Temporal profile and determinants of viral shedding and of viral clearance confirmation on nasopharyngeal swabs from SARS-CoV-2-positive subjects: a population-based study in Reggio Emilia ", the authors determined the timing of viral clearance (first negative RT-PCR on nasopharyngeal swab) and the probability of viral clearance confirmation (two consecutive negative swabs) in COVID-19 patients. This prospective cohort study, recruiting 1162 patients, describes the time characteristics of virus clearance and found one in five false negative tests. This study also revealed that the time to viral clearance increased with age and disease severity.
The main limitation of this current study was that a few of covariates were included in multivariate logistic regression model, while some important indicators, such as previous complications, laboratory tests, and treatment, were not collected. Therefore, the risk factors derived from this model may not be comprehensive.

RE: We agree with the reviewer that few covariates were included in our study. A limitation of population-based studies is that, generally speaking, less clinical information on included subjects is

available, especially for non-hospitalised and paucisymptomatic patients. Nevertheless, the aim of our study was to describe the distribution of time to negative RT-PCR to support the decision making in the follow-up protocol. Thus, we were more interested in understanding and describing whether testing protocols should be based on major determinants rather than in identifying clinical risk factors of prolonged viral shedding. From this point of view, as we can conclude that medians were quite similar even in very different patients, there is no need to tailor the time of testing for negativization. We have added a comment in the Discussion (see page 15, Implications for practice).

Overall, the manuscript is concisely organized and written well. The following comments should be considered.

1. The authors described that “Death occurs in about 20% of cases…”. However, data from ECDC (reference 5) as of 25 March showed that Among hospitalized cases, severe illness was reported in 15% of cases, and death occurred in 12% of these cases. Which area does the high mortality rate of 20% refer to?

RE: We thank the reviewer for this comment. In the study period, the case fatality rate was 20% in Emilia-Romagna region (Girogi rossi JMV J Clin Virol. 2020;128:104415. doi:10.1016/j.jcv.2020.104415) as well as in many other regions in Italy (Riccardo et al medRxiv 2020.04.08.20056861; doi: https://doi.org/10.1101/2020.04.08.20056861). The assessment of case fatality rate by routine statistical analyses may be limited by the cross-sectional nature of the surveillance data as cases were not followed up for a sufficient length of time to observe the entire duration of the disease until recovery or death. We have included the geographical area and we have added references in the Introduction. Thank you. (see page 5, Introduction)

2. Data analysis showed that the time to viral clearance increased with age and disease severity. However, these two indicators are closely related according to previous studies. Multivariable regression showed increasing odds of in-hospital death associated with older age (odds ratio 1·10, 95% CI 1·03-1·17, per year increase; p=0·0043) in Zhou F’s study. (Lancet. 2020;395(10229):1054-1062.)

RE: We thank the reviewer for this comment. Indeed, age and severity of disease for COVID-19 are strongly related. As the aim of our study was to describe the time to viral clearance, we did not assess the causality influencing this distribution. Nevertheless, we performed median time by disease severity adjusted by age and we found no differences compared to the unadjusted trend. In the revised version we reported that the trend is confirmed when adjusting by age in the results section (see page 9, Statistical analysis; see page 11 Results).

It is worth noting that, even if we found slight differences in median time, our data suggest that that a recommendation for tailored surveillance based on reported characteristics is not warranted since differences between groups will not substantially increase testing efficiency. The message we want to convey is that if we want to observe negativization, tests should be performed at least more than 30 days after symptom onset. In the new version, we state this in the Discussion as well. Thank you for your comment, which has improved our manuscript. (see page 15, Implications for practice)

VERSION 2 – REVIEW

REVIEWER	Aijun Pan The First Affiliated Hospital of USTC (Anhui Provincial Hospital), China
REVIEW RETURNED	15-Jul-2020

GENERAL COMMENTS	The author has made reasonable revisions to the article and I think the publication requirements have been met.