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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

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ABSTRACT

Objectives To determine 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 and to identify related determinants.

Design Population-based prospective cohort study on archive data.

Setting Preventive services and hospital care in the Reggio Emilia province, northern Italy.

Participants All 1162 subjects testing positive to RT-PCR on nasopharyngeal swabs and diagnosed with COVID-19 in the Reggio Emilia province with at least 30 days of follow-up by 22 April 2020.

Main outcome measures Median times from diagnosis and from symptom onset to viral clearance with IQR assessed using the Kaplan–Meier estimator, stratified by included characteristics. The probability of viral clearance confirmation, stratified by time from diagnosis and putative determinants assessed using a multivariate logistic regression model.

Results Viral clearance was achieved by 60.6% (704/1162) of patients, with a median time of 30 days from diagnosis (IQR 23–40) and 36 days from symptom onset (IQR 28–45). Of those negative and retested, 78.7% (436/554) had viral clearance confirmation, suggesting one in five false negative tests. The time from symptom onset to viral clearance slightly increased with age, from 35 (IQR 26–44) days under age 50 to 38 (IQR 28–44) in over age 80, and with disease severity, from 33 (IQR 25–41) days in non-hospitalised subjects to 38 (IQR 30–47) days in hospitalised patients. The probability of confirmed viral clearance reached 86.8% after 34 days from symptom onset and increased with time, even when adjusting for age and sex (OR 1.16 95% CI 1.06 to 1.26 per day from diagnosis).

Conclusions Postponing follow-up testing of clinically recovered COVID-19 patients could increase the efficiency and performance of testing protocols. Understanding viral shedding duration also has implications for containment measures of paucisymptomatic subjects.

Strengths and limitations of this study

- This is one of the few studies providing population-based evidence on the duration of viral shedding in SARS-CoV-2-positive patients.
- All patients testing positive for SARS-CoV-2 in the province of Reggio Emilia with a minimum follow-up of 30 days up to 22 April 2020 were included.
- The median time from symptom onset and from diagnosis to viral clearance (ie, first negative RT-PCR assay on nasopharyngeal swab) was assessed and stratified by putative determinants.
- The assessment of time to viral clearance was limited by the testing intervals, which in our study reflect the real-world practice.
- The probability of confirmation of viral clearance (ie, two consecutive negative swabs) was also reported overall and stratified by time from diagnosis and symptom onset.

INTRODUCTION

The worldwide pandemic caused by the new coronavirus SARS-CoV-2, the virus causing COVID-19, has posed enormous challenges to current and future healthcare systems and to governments.[1 2] Italy was the first developed country to have high local transmission: by the middle of April 2020, it ranked third worldwide in terms of the number of cases and of disease-related deaths.[3 4]

COVID-19 presents clinically with a wide range of symptoms, from none at all to severe interstitial pneumonia and systemic

alterations linked to the inflammatory response. Up to April 2020, in the Emilia-Romagna region, as in many other Italian regions, death occurred in about 20% of cases,[5 6] with large differences by sex and age.[7 8]

Although no treatment or vaccine has yet proven to be effective, progress has been made in understanding the pathological processes triggered by the virus and the body's response.[9] This has made it possible to identify populations at higher risk[8 10 11] and to introduce a number of drugs to manage COVID-19 patients that are currently being tested in many national and international trials.[12]

With regard to controlling the spread of the disease, the contact tracing efforts of public health departments (PHD) and the social distancing measures progressively implemented by the Italian Government starting on 8 March 2020, have both played an important role.[13–15] Thanks to these measures, a reduction in the number of new cases was seen by the second half of April. Planning the start of Phase 2, which will entail a gradual reopening, has now begun.[16]

During Phase 2, PHD containment activities will become even more crucial in preventing new outbreaks. These activities will take place alongside the follow-up of current cases. On 22 April 2020, there were more than 23 000 cases in the Emilia-Romagna region.[16 17] Until there is information on whether patients who are clinically recovered but still swab-positive are contagious,[18] and given the low sensitivity of viral tests,[19–21] patients must remain isolated until complete viral clearance is achieved as confirmed by RT-PCR for SARS-CoV-2 on two consecutive negative swabs.[22]

As this will result in an enormous increase in the workload of national healthcare services, knowing as much as possible about the distribution of clearance timing and its determinants is fundamental to optimising the number of tests necessary to obtain viral clearance confirmation and thus to reducing the amount of time in unnecessary isolation of those patients who are clinically recovered. Given that relatively little time has passed since the epidemic began, this information is not yet available in the literature.

The primary aim of this cohort study was to describe the distribution of the timing from diagnosis of COVID-19 and from symptom onset to a negative nasopharyngeal swab, assessing possible determinants of the duration of positivity.

The secondary aim was to assess the probability of viral clearance confirmation (two negative swabs) and its possible determinants.

METHODS

Study design

Prospective population-based cohort study on routinely collected data.

Setting and study population

The study included all COVID-19 patients, that is, symptomatic individuals whose nasopharyngeal swab was positive on RT-PCR, with a diagnosis between 26 February and 22 April 2020, and resident in the Reggio Emilia province.

The province of Reggio Emilia, with a population of over 532,000, is located in the Emilia-Romagna region, one of the three Italian regions most affected by the pandemic as of the middle of April 2020, with an infection rate of about 7/1000.[17]

Of the 4538 residents of the province who tested positive as of 22 April 2020, all those for whom the date of the first positive swab was available and who had had at least 1 day of follow-up were included in a preliminary analysis (n.4480; 98.7%).

Included in the main analysis, instead, were those positive cases who were followed up for at least 30 days (diagnosis before 22 March 2020) and who were symptomatic (total: 1162 patients, 94.2% of all positive cases in the same period).

Data sources

Data concerning the results of the RT-PCR assay for SARS-CoV-2 on nasopharyngeal swabs were extracted from the COVID-19 database created by the Azienda USL-IRCCS di Reggio Emilia Information Technology Service. This database uses information flows from the laboratories where analyses are performed, from electronic hospital records and from the Public Health Service.

This database collects all the sociodemographic information on tested subjects as well as information on emergency department (ED) access, hospitalisations and deaths.

The date of symptom onset was collected from ED records, from epidemiological studies of positive subjects and from medical referral forms for nasopharyngeal swabs.

In-hospital deaths due to COVID-19 were recorded in the electronic hospital records, while those occurring in non-hospital settings were certified by the coroner in the municipality where death occurred. In both cases, all deaths were included in the electronic medical records the Public Health Service keeps of COVID-19 patients.

Nasopharyngeal swabs were analysed with RT-PCR assay at accredited laboratories in the provinces of Reggio Emilia, Parma and Bologna, all in the Emilia-Romagna region.

RT-PCR methodology

For molecular diagnosis of SARS-CoV-2 infection, both nasopharyngeal and oropharyngeal swabs were collected, combining them in a single tube to maximise test sensitivity and to limit the use of testing resources in accordance with CDC guidelines.[23] Samples were collected using flocked swabs to increase the collection of viral load and release of cellular material, and were preserved in a single sterile tube containing viral transport medium

(Copan UTM). Refrigerated samples were sent to the laboratory within 24 hours from sampling.

A commercial one-step reverse transcription real-time polymerase chain reaction (Allplex 2019-nCoV Assay, Seegene) was performed to confirm the presence of SARS-Cov-2 by amplification of RdRp, E and N gene in the swab specimens of patients according to the Corman protocol with a limit of detection of 100 copies of RNA/reaction. Nucleic acid extraction and PCR setup was performed by the Microlab NIMBUS system. RT-PCR assay was performed on a CFX96 real-time PCR Detection System (CFX Manager Software-IVD v1.6) (Bio-Rad).[24]

Outcome measures

The main outcome was viral clearance as determined by RT-PCR negativity on one nasopharyngeal swab.

The secondary outcome was viral clearance confirmation as determined by RT-PCR negativity on two consecutive nasopharyngeal swabs. One minus proportion of clearance confirmation can be interpreted as a proxy of the probability of a false negative result of the viral test.

Follow-up

The start of follow-up is determined by the date of the first positive nasopharyngeal swab.

The main outcome was assessed by considering the first negative nasopharyngeal swab, performed at least 10 days after the first positive swab. This 10-day period was selected so that hospitalised patients did not undergo repeated swabs to monitor disease. The Italian monitoring protocol of positive cases calls for negativity testing to be performed no earlier than 14 days after the first positive test and at least 3 days after symptoms have disappeared. If the test is positive, a swab is repeated after at least 7 days, while if negative, a second swab is done after at least 48 hours to confirm viral clearance.[22]

The secondary outcome was assessed by including only those patients who had had at least one negative swab at least 10 days after the first negative swab. The proportion of negative second swabs was quantified for this subcohort of patients.

The end of follow-up was defined as the date of the main outcome, with the date of death or the date of the end of follow-up (22 April 2020).

The timing of viral clearance was assessed starting from the date of the first positive swab and, in the secondary analysis, starting from the date of symptom onset.

Covariates

Each patient's main sociodemographic characteristics (sex, age, citizenship) were included in the analyses. Clinical data were not available for all included cases since not all were hospitalised. Thus, ED access and hospitalisation were used as proxies of disease severity.

Statistical analyses

Descriptive analyses of the patients in the cohort are reported, including the rate of viral clearance for each covariate considered.

The probability of viral clearance confirmation was calculated overall, stratified by each included covariate and for time from first positive swab.

Median times to viral clearance were also calculated, with 25th and 75th percentile (IQR), from the first positive swab and from symptom onset, overall and stratified by the covariates considered, by estimating survival using the Kaplan–Meier estimator. Median times to viral clearance for disease severity adjusted by age were also calculated. Finally, a multivariate logistic regression model was used to assess the impact of determinants included in the study on the probability of negativity confirmation (sex, age, time to viral clearance), calculating the OR and relative 95% CI .

Patient and public involvement

This research was done without patient and public involvement. Despite this, the study authors agree to consider research on COVID-19 a current priority also from patients' and public perspectives.

Ethical aspects

The study was approved by the Area Vasta Emilia Nord Ethics Committee on 7 April 2020, protocol n.2020/0045199.

Patient consent

In accordance with the Italian privacy law, no patient or parental consent is required for large retrospective population-based studies approved by the competent Ethics Committee if data are published only in aggregated form.

Funding

The study has been conducted using exclusively institutional funds of the Azienda USL-IRCCS di Reggio Emilia. There was no external funding source for this study.

RESULTS

From 27 February to 22 April 2020, 4538 residents in the Reggio Emilia province tested positive for SARS-Cov-2 on RT-PCR assay performed on nasopharyngeal swab. The date of positive swab was not available for 21 of these individuals and 37 had less than 1 day of follow-up.

In the same period, of the remaining 4480 subjects, 1259 achieved viral clearance (at least one negative swab following the initial positive swab) and 428 died, for a total of 465 deaths (10.2%).

The median time to viral clearance, estimated by the Kaplan–Meier estimator, was 31 days from first positive swab (IQR 24–41) (online supplemental material, figure 1).

From this population, the 1162 patients who were diagnosed before 22 March were selected to permit a detailed assessment of patients who had had a follow-up of at least 30 days and for whom the date of symptom onset was available.

Table 1 Sociodemographic characteristics and disease severity, median number of nasopharyngeal swabs per subject with RT-PCR assay for SARS-CoV-2, number (and proportion) of subjects with at least one negative nasopharyngeal swab and number (and proportion) of these subjects who received viral clearance confirmation on retesting in the cohort of subjects resident in the province of Reggio Emilia and diagnosed with COVID-19 before 22 March 2020 and followed up until 22 April 2020

		Total swabs per subject	First negative swab	Confirmed negative swab
	Overall sample tested N (%)	(median, range)	N negative (%)	N° confirmed / N° retested (%)
Total	**1162**	**3 (1–9)**	**704 (60.6)**	**436/554 (78.7)**
Deaths	172 (14.8)			
of which death before start of follow-up	110			
Sex				
M	652 (56.1)	3 (1–8)	394 (60.4)	257/321 (80.1)
F	510 (43.9)	3 (1–9)	310 (60.8)	179/233 (76.8)
Age Mean (SD)	60.7 (16.3)			
Age categories				
<50	303 (26.1)	4 (1–8)	215 (71.0)	147/176 (83.5)
50–59	258 (22.2)	3 (1–8)	184 (71.3)	113/151 (74.8)
60–69	229 (19.7)	3 (1–9)	154 (67.2)	89/120 (74.2)
70–79	193 (16.6)	3 (1–8)	98 (50.8)	57/72 (79.2)
≥80	179 (15.4)	2 (1–7)	53 (29.6)	30/35 (85.7)
Citizenship				
Italian	1108 (95.4)	3 (1–9)	667 (60.2)	418/530 (78.9)
Foreign	54 (4.6)	3 (1–7)	37 (68.5)	18/24 (75.0)
Disease severity				
No access to Emergency Department or hospital	353 (30.4)	4 (1–9)	271 (76.8)	174/223 (78.2)
Emergency Department use only	232 (20.0)	3 (1–7)	155 (66.8)	96/124 (77.4)
Hospitalisation*	577 (49.6)	3 (1–8)	278 (48.2)	166/207 (80.2)

*Hospitalisation excluding patients using Emergency Department only AND patients with no hospitalisation and no Emergency Department use.
ED, Emergency Department.

Characteristics of this subcohort are described in table 1. Viral clearance of this population was assessed from day 10 after the first positive swab.

Of the 1162 patients included in the study, 110 died in the first 9 days after the positive swab and were therefore not included in the follow-up to calculate the median time to viral clearance. Sixty-two others died during the study period, for a total of 172 deaths (14.8%). Of the 577 hospitalised patients, 170 (mean age 79.1 years, SD 10.4) died in the study period (29.5%). Of the 232 subjects that accessed the ED but were not hospitalised, two deaths occurred (0.9%; mean age 79.5 years, SD 14.8), while no deaths occurred in those not seeking hospital care.

Viral clearance

During follow-up, each patient underwent an average of three swabs, with a range of from one to nine (online supplemental material, table 1). The mean time of retesting after positive swabs was 14.7 days (SD 10.4) after the first positive, 14.0 days (SD 8.0) after the second positive and 9.2 days (SD 4.1) after the third positive swab.

Viral clearance was detected in 704 of the 1162 patients (60.6%) and confirmed in 78.7% of those who underwent a second test after the first negative swab (436/554), which suggests that there was about one false negative for every five negative results. These results, stratified by demographic characteristics and disease severity, are reported in table 1.

Median time to viral clearance in this cohort was 30 days from the first positive swab and 36 days from symptom onset, with an increasing trend for increasing age and disease severity as assessed by ED access or hospitalisation (table 2). The increasing trend by disease severity remained after adjusting for age, rising from 28 (IQR 20–36) to 31 (IQR 24–41) when considering days since diagnosis and from 32 (IQR 25–41) to 38 (30-47) when considering days since symptom onset.

Table 2 Median time to viral clearance (first negative swab on RT-PCR for SARS-CoV-2) and IQR from diagnosis and from symptom onset by covariates calculated using Kaplan–Meier survival estimator on 1162 patients diagnosed before 22 March 2020, resident in the Reggio Emilia province, and followed up to 22 April 2020

	Time to viral clearance (first negative swab)			
	From first positive swab		From symptom onset	
	Median	25%–75%	Median	25%–75%
Total	30	23–40	36	28–45
Sex				
M	28	21–39	35	27–45
F	31	25–40	37	29–45
Age categories				
<50	29	22–40	35	26–44
50–59	28	21–39	35	27–45
60–69	29	22–39	36	29–43
70–79	33	25–44	39	32–50
≥80	31	24 N.A.	38	28–44
Citizenship				
Italian	30	23–40	36	28–45
Foreign	28	23–39	32	26–45
Disease severity				
No ED use No hospitalisation	28	21–37	33	25–41
ED use only	29	23–42	36	29–47
Hospitalisation*	32	25–42	38	30–47

*Hospitalisation excluding patients using Emergency Department only AND patients with no hospitalisation and no Emergency Department use.
ED, Emergency Department.

Confirmation of viral clearance

The proportion of viral clearance confirmation increased as time intervals increased, with a reduction in false negatives when the first negative swab was performed more than 34 days after the first positive swab (table 3).

A longer interval to first negative swab was significantly associated with a reduction in false negatives even when adjusting for sex and age. Females seemed to have a lower probability of viral clearance confirmation, although this was not statistically significant. (table 4)

DISCUSSION

Principal findings

In the Reggio Emilia cohort, 60.6% of positive SARS-CoV-2 cases diagnosed before 22 March achieved viral clearance, measured as first negative swab, by 22 April 2020. Median time to viral clearance was found to be 30 days from diagnosis and 36 days from symptom onset, with a trend that increased with increasing age and that was slightly longer in hospitalised patients, suggesting that clearance was slower in the more severe cases.

About one fifth (21.3%) of viral clearances in the follow-up period were not confirmed by the second swab, suggesting that there was a high rate of false negatives in this population.

The percentage of confirmed viral tests increased significantly as the interval between the first positive swab or symptom onset and the first negative follow-up swab increased. This result confirms the predictions of a model built based on the results of a number of reports on clearance.[19]

Strengths and weaknesses of the study

It must be noted that the endpoint of viral clearance can only be observed at the moment of testing, a negative swab does not tell us when clearance actually occurred, meaning that we only have a terminus ante quem. The longer the interval between tests, the greater the overestimation of time to clearance.

In this study it was not possible to assess the sensitivity of RT-PCR. Nevertheless, we considered the occurrence of a positive test after a negative one as a proxy of a false negative result, even if we could not exclude that some negative tests followed by a negative confirmation test might have been false negative results as well. However, repeated tests for SARS-CoV2 RT-PCR has been

Table 3 Probability of viral clearance confirmation (two negative nasopharyngeal swabs) by time from diagnosis or symptom onset and first swab negative on RT-PCR for SARS-CoV-2 in a cohort of COVID-19 subjects diagnosed before 22 April 2020 and resident in Reggio Emilia province

Time to first negative from	N° of first negative test	N° of first negative test with retest (and %)	N° of confirmed negative test (those with a negative retest) (and %)
First positive			
10–14 days	34	30 (88.2)	14/30 (46.7)
15–19 days	134	132 (98.5)	95/132 (72.0)
20–24 days	131	124 (94.7)	99/124 (79.8)
25–29 days	196	169 (86.2)	140/169 (82.8)
30–34 days	137	75 (54.7)	67/75 (89.3)
>34 days	72	24 (33.3)	21/24 (87.5)
Symptom onset			
10–14 days	9	7 (77.8)	3/7 (42.9)
15–19 days	23	23 (100.0)	11/23 (47.8)
20–24 days	88	88 (100.0)	66/88 (75.0)
25–29 days	147	137 (93.2)	106/137 (77.4)
30–34 days	158	140 (88.6)	112/140 (80.0)
>34 days	279	159 (57.0)	138/159 (86.8)

considered an acceptable reference standard in previous studies and systematic reviews.[25 26]

The testing protocol in this study was consistent with those recommended by ECDC, with a longer interval only for the second retesting due to the healthcare system overload yet reflecting real-world practice, including outpatient data.

As this was a population-based study, clinical information was not available of all included subjects. We therefore considered access to the ED and hospitalisation as proxy of disease severity. Even if this could limit the accuracy of disease severity assessment, the distribution of deaths in the three groups confirmed a strong association between hospitalisation and the probability of dying of COVID-19 in our cohort. Moreover, defining groups by healthcare service use was more appropriate to support public health decision making since this easily available information could be used to organise testing schedules.

Table 4 Multivariate regression model of viral clearance confirmation including all subjects with a first nasopharyngeal swab negative for SARS-CoV-2 assessed by RT-PCR and retested (n=554) before 22 April 2020. Patients were included if diagnosed before 22 March 2020, and resident in the Reggio Emilia province

Viral clearance confirmation	OR	P-value	95% CI
Time to first negative swab (days)	1.16	0.00	1.06 to 1.26
Sex			
M	1		
F	0.43	0.08	0.17 to 1.09
Age	0.99	0.49	0.98 to 1.01

CI, Confidence Interval; OR, Odds Ratio.

Comparison with other studies and interpretation

The median time to viral clearance observed in our cohort was longer than that reported by two cohort studies of hospitalised patients in Wuhan, China, both of which had a follow-up of about 1 month. The first, which involved 191 subjects, reports a median of 20 days in survivors (IQR 17.0–24.0), with a maximum of 37 days from symptom onset. This study does not report any difference between patients undergoing antiviral therapy with lopinavir/ritonavir. However, longer intervals were observed in patients with more severe disease, as our results also suggest.[27] As the inclusion criterion in this study was hospital discharge between 29 December 2019, and 31 January 2020, however, it is not clear whether patients with longer disease duration may have been excluded.

The second study reports a median of 23 days (IQR 18–32 days) between symptom onset and viral clearance. However, the median was calculated only for those patients who had had two consecutive negative swabs during follow-up (120/168, 71.4%). This way of estimating the median time may lead to an underestimation if the actual number of cohort subjects truly followed up is not taken into consideration. Further, the study reports that 86.7% of the 120 included subjects achieved viral clearance within 37 days of follow-up but that 10 subjects (8.3%) were still positive by day 40.[28] This study also observes an increase in time to viral clearance with greater disease severity, with increasing age and in the absence of antiviral therapy.[28]

A recent case report states that viral shedding was detected up to 49 days from symptom onset.[29]

As Atkinson and Petersen discuss, to be able to use these results to make public health decisions, it must be remembered that RT-PCR can identify even fragments of the virus, meaning that subjects who do not have any active replication and are thus not infectious will nevertheless test positive.[18]

A number of studies have assessed the viral load in SARS-CoV-2-positive subjects in various biological matrices, reporting consistent results. These results describe a period of high viral load in the respiratory airways and, presumably, high transmissibility, starting about 3 days after symptom onset,[25] with a peak in viral load identified between the day before and 4 days after symptom onset and a decrease in load starting from day 8 after symptom onset.[30–33]

Various studies have, however, detected a viral load 20–28 days from symptom onset,[30–34] even when the

virus itself was at times undetectable in the same period, reporting fluctuating results when the viral load approched the limit of detection of diagnostic systems.[31 33]

The half-life of up to 3 months of respiratory epithelial cells and the detectability of genetic materials from a live virus or even from fragments of dead virus by RT-PCR should be also considered to understand the inconsistency in negative results over a prolonged period.[35] After a phase of active viral replication, estimated in 8 days from symptom onset,[33] the persistence of dead virus fragments at concentrations close to the limit of detection could explain the unconfirmed negative test rate in the first weeks after clinical recovery.[35]

Some authors have shown that late positive samples have low viral load and scarce ability to infect cells in vitro,[33 35] suggesting a low, if any, potential for generating new infections. Based on this, the WHO changed the recommendations to discontinue transmission-based precautions for COVID-19 patients.[36] However, virological and epidemiological evidence on the risk of transmission during the convalescent phase characterised by positive RT-PCR is weak, and current serological data have not provided any additional insight.[37] Furthermore, current epidemiological evidence of transmission has been influenced by how quarantine has been managed thus far.

The results concerning the differences in viral load in terms of disease severity are partially discordant.[30–32 34] One study on 3497 samples of different biological matrices from 96 patients admitted to the hospital in Zhejiang, China, found different distributions of viral load in moderate and in severe cases, with a median time to viral clearance on samples taken from respiratory airways of 14 and 21 days, respectively, and a peak in the second week after symptom onset in patients with moderate disease and in the third week in patients with more severe disease. The authors also report longer viral persistence in patients over age 60 and in males.[34] The median time to viral clearance in this study is shorter than that which we observed in our cohort, however, the inclusion criteria were also different, with only cases reaching a negative swab included, and testing was done much more frequently than in our cohort.

Other studies, instead, do not report any differences in viral load between symptomatic and asymptomatic subjects.[4 32 38] Further, from the study on the entire population of the municipality of Vò Euganeo, 43.2% of the subjects who tested positive to SARS-CoV-2 were asymptomatic, and from the reconstruction of the chain of disease transmission, two of the eight new cases observed during follow-up had had contact only with asymptomatic subjects.[38]

These results have important implications for policies of tracing and isolation: they suggest the possibility that asymptomatic and pre-symptomatic subjects are as infectious as symptomatic subjects are, although perhaps for not as long.[4 30–32 38]

Implications for practice

Our data indicate that testing at 14 days from diagnosis, as many regional surveillance protocols recommend, will result in most cases still being positive. So that at least half of these tests are negative, testing should be done after more than 4 weeks once patients are symptom-free. What's more, given the high probability a priori of viral persistence, negative tests 3 weeks from diagnosis have a high probability of being false negatives.

Second, our data suggest that recommendation for tailored surveillance based on age, sex and disease severity of each patient is not warranted, since median times are quite similar even in very different patients, and personalised time for retesting would not increase surveillance efficiency more than would an overall delay in start of testing.

A third important implication of our results for practice concerns the management of isolating and monitoring paucisymptomatic suspected COVID-19 subjects who have not been tested due either to our health services' difficulty in performing the test at home during the most impactful phase of the epidemic or because not enough tests were available. At the moment, paucisymptomatic subjects receive the recommendation to self-isolate during the symptomatic phase, but there are no clear indications on what to do once symptoms have disappeared. If these subjects have indeed been infected with SARS-2-CoV, all the evidence suggests that viral clearance even in them will not be achieved rapidly. To avoid generating secondary cases, either the isolation period should be longer (over 30 days from symptom onset) or at least one follow-up test should be done before ceasing isolation.

Finally, our results point out that almost all asymptomatic COVID-19 patients and a large proportion of symptomatic patients who will be eligible to discontinue transmission-based precautions (including isolation) according to the most recent WHO recommendations of 27 May 2020[36] will test positive for SARS-Cov-2 on RT-PCR when released. Since there is still uncertainty regarding whether these same patients are infectious, our results have relevant public health implications.

Dissemination declaration

We plan to disseminate the results to patient organizations through internal reports and peer-review manuscript, when available, translated in original language.

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