

From positive to negative: a time to event analysis in Regione Lombardia.

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Abstract

Objectives. To assess the time span from positive to negative SARS-CoV-2 RNA detection by RT-PCR, and to evaluate the reliability of the test-based criteria as the required condition for the reintroduction of the asymptomatic SARS-CoV-2 positive patient in the community.

Methods. We used information concerning negativization and the respective times. Cumulative probabilities of negativization during the follow-up were evaluated by through Crude Cumulative Incidences (CCIs). Non-parametric estimates of CCIs and respective 95% C.I.s were obtained.

Results. We report only the results for 52,186 individuals. 33486 subjects resulted negative or potentially negative with a CCI of 75.2% at 70 days from the first swab (95% CI: 74.8% to 75.7%). 11,000 subjects deceased before 14/05/2020 without diagnosis of negative status (CCI 21.9%; 95% CI: 21.5% to 22.3%) at 56 days from the first swab (maximum observed time to death).

Conclusions. SARS-CoV-2 positivity is a condition that frequently lasts more than 30 days. Since isolation based only on positivity status could be excessive, more solid studies are required to determine a single internationally accepted policy regarding the dismissal of quarantine and isolation.

Introduction

In December 2020, in China, a novel strain of coronavirus was recognized to be the infective agent causing an abnormal peak of atypical pneumonia (1). The 31st of December 2020 marks the official date of the arrival of the virus in Italy, as two Chinese tourists were first diagnosed positive to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2) and then admitted to Spallanzani hospital in Rome. On the 20th of February in Codogno (LO) the first Italian patient positive to Sars-nCov-2 was hospitalized (3).

SARS-CoV-2 belongs to the family of coronavirus, targets the airways, and is incubated for a time span that goes from 5 to 14 days (5). Common symptoms are anosmia, dysgeusia, cough, fever, diarrhea, nausea, vomit, dyspnea (6). Severity of symptoms can widely vary but they are not always present: a study in Vò Euganeo (Italy) showed that out of the total population that tested positive, around 40% of them were asymptomatic (7).

The Real Time – Polymerase Chain Reaction (RT-PCR) analysis run on nasopharyngeal swab is the gold standard for the diagnosis of Coronavirus infection disease (COVID-19) (8). It detects fragments of viral RNA on the upper airways regardless of the viral load. Up to now, a patient is considered to be positive to SARS-CoV-2 depending only on the positive result of the nasopharyngeal swab, regardless of symptoms (9). According to the Italian national guidelines, individuals that came in contact with a case of COVID-19 should be quarantined for 14 days and one PCR test should be performed at the end of the quarantine time if no symptoms have been developed meanwhile. If the individual becomes symptomatic he will be isolated and a nasopharyngeal swab will be performed immediately and quarantine and isolation will be continued until at least the end of symptoms. Isolation and quarantine will only be discontinued after two negative results of RT-PCR. (10)

Recovery is controversial: both Center for Disease Control (CDC) and World Health Organization (WHO) discard a test-based criterion for the termination of isolation for patients not in healthcare settings, only relying on temporal criteria (11) that changes depending on the presence of symptoms. For the Italian Ministry of Health a symptomatic patient is considered “recovered” after the complete resolution of symptoms, and when two swabs, performed 24 hours apart from each other, test negative (12), while for patients that never developed symptoms one negative swab after the 14 days of quarantine is enough to go back in the community.

The aims of this study are the assessment of the time span during which a patient diagnosed positive to COVID-19 becomes negative (“negativization time”) to viral RNA detection by RT-PCR, and the evaluation of the reliability of the test-based criteria as the required condition for the reintroduction of the asymptomatic SARS-CoV-2 positive patient in the community.

We used a large dataset relative to the outbreak phase, including the earliest period of virus spread and the subsequent phase of Public Health Emergency. In this study we performed a retrospective reconstruction of the negative status resulting at the date of 14/05/2020 (end of study) of subjects diagnosed as “COVID-positive” in Lombardy, and of the time elapsed from the first diagnosis to the date in which that status was ascertained.

In the emergency period a drastic rise of mortality has been experienced in Lombardy, especially in the elder population. For those inhabitants who deceased before the end of study without a confirmed negative status, it was not possible to know their negativization times. This situation is named as “presence of competing risks”, therefore a specific method of statistical analysis was adopted for accounting for this issue.

Methods

Lombardy region collected the data of analysis by combining the information retrieved from two sources: the list of SARS-CoV-2 positive patients derived from contact tracing activity and the list of positive patients derived from the laboratories that performed SARS-CoV-2 diagnosis by RT-PCR on nasopharyngeal and oropharyngeal swab. The data were then integrated with the information contained in the database dedicated to the comorbidities and to the personal data so as to elaborate a single database collecting all the SARS-CoV-2 positive patients, the first positive diagnostic swab and, when performed, the double negative swab testifying the resolution of the disease.

In this work we used only information concerning the negativization and the respective times. Data consist of the dates of delivery of samples to laboratory for 229,565 swabs performed in Lombardy region from 20/02/2020 to 14/05/2020 (follow-up period) on 81,963 subjects with positive diagnosis of COVID-19, and of subject’s demographic characteristics - gender and age – and date of decease. The endpoint was, for each subject, the time elapsed from the date of delivery of the first swab with positive result and the date of delivery of the last swab. Subjects resulting negative by last swab’s results were classified either

as negative to COVID-19 if there was an additional negative swab delivered no more than 24 hours earlier; or, otherwise, as potentially negative to COVID-19. Decease occurred before the end of study without a negative status was considered as competing event. Furthermore, subjects alive and resulting positive to COVID-19 by the last swab were considered as subjects with censored status at the end of follow-up (14/05/2020).

Cumulative probabilities of negativization during the follow-up were evaluated by through Crude Cumulative Incidences (CCIs). Non-parametric estimates of CCIs and respective 95% C.I.s were obtained using the method described by Kalbfleisch and Prentice (13). The analysis was performed both for the overall collective and by stratifying subjects by gender, and by age classes (0-19, 20-34, 35-49, 65-79, 80-120 years).

Results

We excluded 10,862 subjects due to incomplete, inconsistent, or potentially inconsistent information that could prevent the correct computation of negativization times. Here we report only the results for 52,186 individuals who received the first diagnosis of positivity COVID-19 at least 30 days before the end of follow-up (14/05/2020). Of these 52,186, 27,002 (51.7%) were female. The 0-19 age group included 365 subjects (0.7%), the 20-34 age groups 3167 subjects (6.1%), the 35-49 age group 7626 subjects (14.6%), the 50-64 age group 13698 subjects (26.2%) the 65-79 age groups 13860 subjects (26.6%). The 80-120 age group 13470 subjects (25.8%).

Thirty three thousand 4 hundreds and eighty-six subjects resulted negative or potentially negative to COVID-19 with a CCI of 75.2% at 70 days from the first swab (95% CI: 74.8% to 75.7%). Of these, 9,570 resulted negative and 23,916 subjects resulted potentially negative. 11,000 subjects deceased before 14/05/2020 without diagnosis of negative status. The corresponding CCI was 21.9% (95% CI: 21.5% to 22.3%) at 56 days from the first swab (maximum observed time to death). The CCIs for the whole population, estimated at weekly intervals, are reported in Table 1. Less than 5% of subjects are estimated to become negative within 2 weeks from the first swab.

Days	DECEASED	NEGATIVE OR POTENTIALLY NEGATIVE
7	13.3 (13.0%, 13.6%)	0.7% (0.7%, 0.8%)
14	18.5 (18.2%, 18.8%)	4.2% (4.0%, 4.3%)
21	20.5 (20.1%, 20.8%)	16.6% (16.3%, 16.9%)
28	21.3 (21.0%, 21.7%)	31.1% (30.7%, 31.5%)
35	21.7 (21.3%, 22.0%)	45.2% (44.8%, 45.7%)
42	21.8 (21.5%, 22.2%)	56.3% (55.8%, 56.7%)
49	21.9 (21.5%, 22.2%)	64.5% (64.1%, 64.9%)
56	21.9 (21.5%, 22.3%)	69.8% (69.4%, 70.3%)
63	-	73.1% (72.7%, 73.6%)
70	-	75.2% (74.8%, 75.7%)

Table 1. Crude Cumulative Incidence (CCI) for the entire cohort. Days = Time from the first swab. Deceased = CCI of death, Negative = CCI of negativization.

As shown in Fig. 1, the CCI for negativity has a slow increase in the first two weeks, then a rapid increase and finally a plateau at around 60 days from diagnosis.

In Supplemental Material we reported the CCI for negativity stratified for sex. It may be seen that, for each week, CCIs of females are higher than CCIs of males.

When stratified by age group the result showed significant differences. Table 2 shows CCI of negativity stratified for age groups. Overall CCIs are similar for age groups 0-19 to 35-39 after 4 weeks from the first swab. In the elder classes (65-79 and 80-120 years old) the CCIs are lower, as compared to the youngest ones, at each week.

Days	AGE					
	0-19 yo (365)	20-34 yo (3167)	35-49 yo (7626)	50-64 yo (13698)	65-79 yo (13860)	80-120 yo (13470)
7	3.0% (1.3%, 4.8%)	1.6% (1.2%, 2.0%)	0.9% (0.7%, 1.1%)	0.7% (0.6%, 0.9%)	0.6% (0.5%, 0.7%)	0.5% (0.4%, 0.6%)
14	9.0% (6.1%, 12.0%)	8.9% (7.9%, 9.9%)	7.1% (6.6%, 7.7%)	5.4% (5.0%, 5.8%)	2.6% (2.3%, 2.9%)	1.5% (1.3%, 1.7%)
21	28.5% (23.9%, 33.1%)	34.2% (32.5%, 35.8%)	28.5% (27.5%, 29.5%)	21.7% (21.0%, 22.4%)	9.3% (8.8%, 9.8%)	7.8% (7.3%, 8.2%)
28	50.4% (45.3%, 55.6%)	54.4% (52.7%, 56.2%)	49.1% (48.0%, 50.2%)	39.9% (39.0%, 40.7%)	20.6% (19.9%, 21.3%)	16.7% (16.0%, 17.3%)
35	65.8% (60.8%, 70.7%)	69.9% (68.3%, 71.5%)	66.4% (65.3%, 67.5%)	57.3% (56.5%, 58.1%)	33.7% (32.9%, 34.5%)	26.5% (25.7%, 27.2%)
42	77.0% (72.6%, 81.5%)	80.4% (78.9%, 81.8%)	78.0% (77.0%, 78.9%)	70.3% (69.5%, 71.1%)	45.1% (44.2%, 45.9%)	34.5% (33.6%, 35.3%)
49	84.4% (80.4%, 88.4%)	87.3% (86.0%, 88.6%)	86.0% (85.1%, 86.8%)	79.4% (78.7%, 80.1%)	53.8% (52.9%, 54.6%)	41.7% (40.8%, 42.7%)
56	89.6% (85.8%, 93.3%)	91.0% (89.8%, 92.2%)	90.7% (89.9%, 91.5%)	85.4% (84.8%, 86.1%)	59.6% (58.8%, 60.5%)	46.4% (45.4%, 47.4%)
63	92.8% (88.9%, 96.7%)	94.2% (93.0%, 95.4%)	93.8% (93.0%, 94.5%)	89.0% (88.4%, 89.7%)	62.9% (62.0%, 63.8%)	49.7% (48.7%, 50.7%)
70	-	-	-	-	65.1% (64.1%, 66.0%)	-

Table 2. CCI for negativity (negative or potentially negative subjects) after stratification for age groups.

Discussion

Since the first outbreak of SARS-CoV-2 infection in China, COVID-19 epidemic spread throughout the world involving more than 37 million people (14). In Italy, the exponential growth of positive cases, especially in the first weeks, brought a rapid succession of Government policies aiming at controlling the spreading of the disease (15). Prevalent cases account for most of the present cases in Italy, therefore one of the most important questions to answer remains the duration of the disease itself.

This research focused on the evaluation of the interval between the first diagnosis of SARS-CoV-2 infection and both the first negative RT-PCR testing and the double negative result that accounts for the recovery. On total population (n=52186) the analysis showed a CCI for negativity (considering both the single last and the double negative sample) of 16.6%, 31.1%, 45.2% and 56.3% at 2, 28, 35 and 42 days from diagnosis respectively.

When the same population is stratified for sex, CCI for women showed a more rapid increase accounting for a higher probability than men of being negative or potentially negative for women, at any time interval. The stratified analysis for age showed a pattern in which younger patients had a consistently higher probability of negative or potentially negative than older patients, especially for higher time intervals. The lowest CCI curve was evident for patients older than 80 yo. As shown in Fig. 2, patients older than 65 yo showed a sensibly lower CCI than any younger age group.

These remarkable differences between age groups are partly motivated by the consistently higher probability of death in these older patients; in fact, CCI refers to the probability that the event verifies as the first event compared to the other events considered, as in this case death.

Patients older than 65 yo showed CCIs for negativity almost halved compared to those of younger age groups, on the contrary, when considering CCI for death, the older age groups showed a significantly higher probability than that of younger age groups.

Our results are in accordance with the work of Mancuso et al. (16) which demonstrated in a sample of 1162 patients that 60,6% of subjects became negative at a median follow up time of 30 days from diagnosis and 36 days from symptoms onset. Moreover, in a recent submitted article, available in pre-print, Lombardi et al. reported a median time from first positive test to a negative test to be 27 days (95% CI: 24-30) (17). The results of our study have been obtained independently of symptoms, therefore the positivity of samples at RT-PCR testing was not related to a clinical correlation and we can't speculate on the probability of positive patients to be contagious.

This study has a few limitations. In the initial phase of the Public Health Emergency we lacked an official testing protocol for SARS-Cov-2 in Italy (18). As a consequence, data have been recorded without a planned national strategy. A second issue is the absence of a rationale, confirmed by reliable study results,

that explains the possibility of occurrence of a positive swab after a first negative result. Thus, the negativity status should be ascertained considering, from the sequence of swabs performed to a single subject, the result of the last one: if the result is negative the subject is considered negative. Although the loss of accuracy, this choice is useful to avoid putative under-estimation of negativization times.

Up to date, SARS-CoV-2 contagiousness has been reported in current literature to be evaluated not only by the positivity to RT-PCR, but also considering the viral replication. In fact, several studies posit that the likelihood of recovering replication-competent virus declines after onset of symptoms. In patients with mild to moderate symptoms, no trace of a replication-competent virus was found after 10 days following symptom onset (19, 20, 21). In patients with severe symptoms, which in some cases were complicated by immunocompromised state, replication-competent virus was isolated between 10 and 20 days after symptom onset (22); even though, 88% and 95% of their biological fluids tested negative for replication-competent virus research after 10 and 15 days, respectively, following symptom onset.

At the same time, it is evident that a high fraction of SARS-CoV-2 positive patients remain positive for a long time span; this implies that, if the test-based criteria is used as the necessary condition to end the isolation, most patients will be isolated for a long time regardless of symptoms resolution.

These considerations need to be done especially due to the impact of containment measures on those activities that would suffer the most from this policy: manufacturing and productive activities, schooling and education. A strict policy of a long quarantine means loss of work hours, and sometimes entire departments will be sent home. The impact of the containment measures will be both short and long time: during the 4th quarter of the 2020 the Gross Domestic Product (GDP) will contract of around 11%, and more than half of it is due to COVID-19 induced uncertainty (23); also, given that every additional year of schooling translates to 8 percent in future earnings, a study demonstrated that the cost of school closures due to earning losses as a percent of GDP will range from 9% in high income countries to 61% in low income countries (24).

The test-based criteria has been discarded by the major scientific organization (WHO, CDC) but it still is the requirements for the community re-admission for many nations. As a consequence, the absence of a single internationally-shared procedure that grants 100% safety causes great uncertainty and confusion, also taking into account that a 60 days long isolation is not easily manageable and maybe not even necessary.

In conclusion, it appears clear that SARS-CoV-2 positivity is a condition that frequently lasts more than 30 days, as we observed in our cohort of patients. To be able to determine the accordance between positivity to the test and being contagious is paramount in order to avoid very long isolation or quarantine which would be unsustainable, but, at the same time, shortening the time span to less than 10-15 days would pose a concrete risk of increasing the virus spreading in the population therefore more solid studies are required in order to determine a single internationally accepted policy regarding the dismissal of quarantine and isolation.

Declarations

Conflict of Interest

The authors have no conflicts of interest.

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Figures

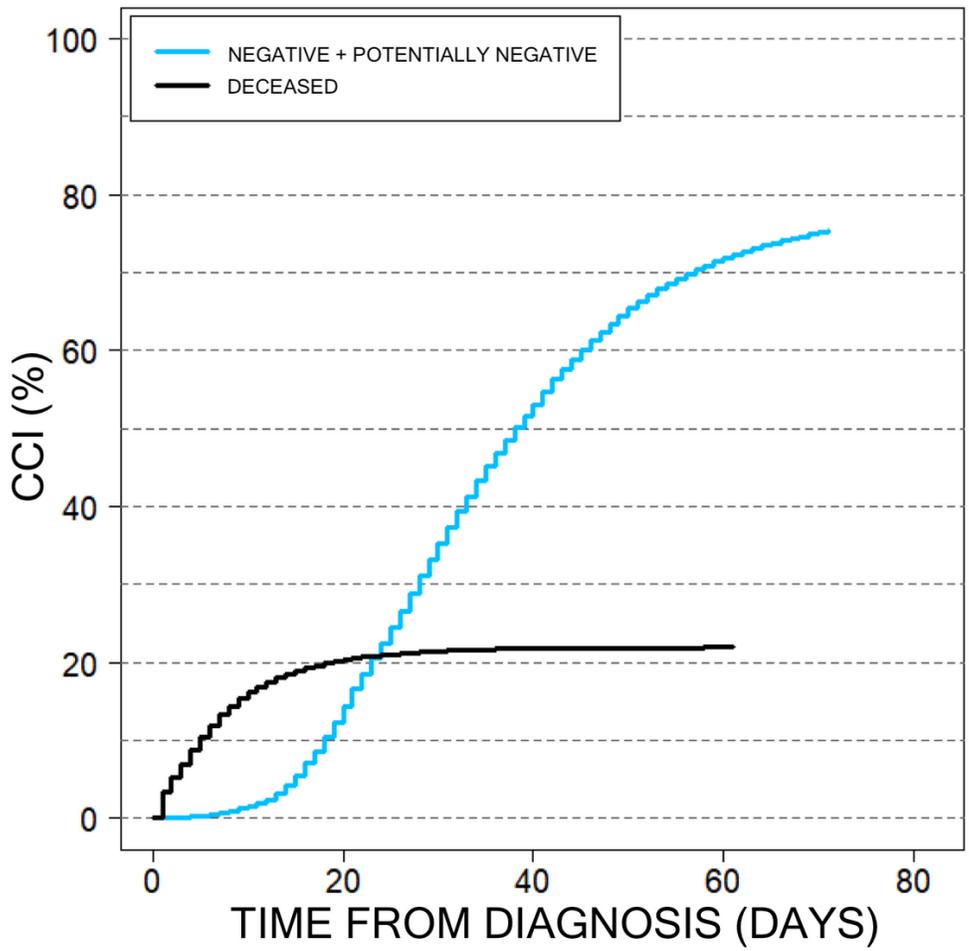


Figure 1

Crude Cumulative Incidence (CCI) for any negativity status and death.

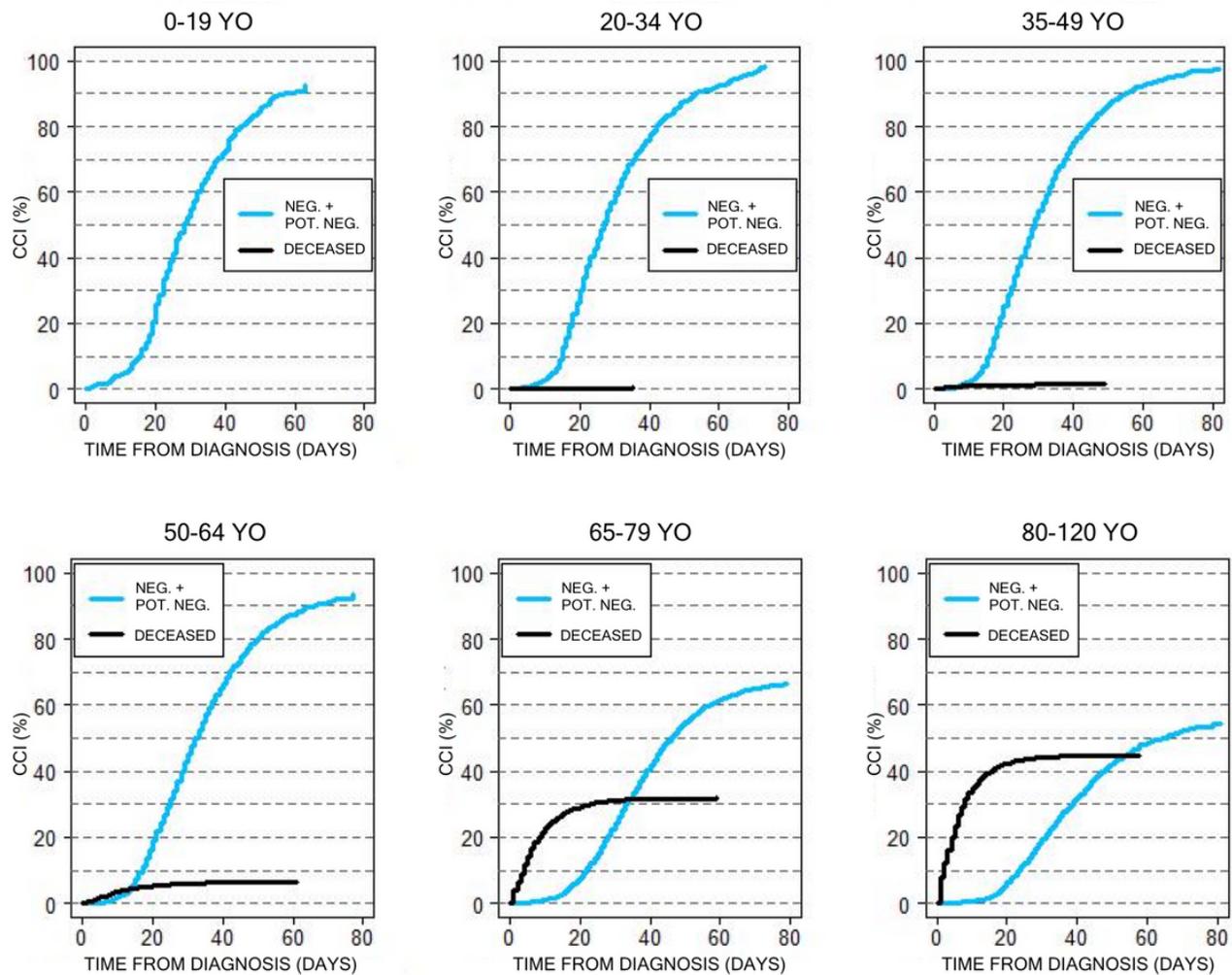


Figure 2

Crude Cumulative Incidence (CCI) for any negativity status and death stratified for age groups.

Supplementary Files

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