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Original Research

False-negative real-time polymerase chain reaction tests in COVID-19 patients: an epidemiological analysis of 302 patients

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ABSTRACT

Objectives: Patients who arrive at the emergency department (ED) with COVID-19, who test negative at the first real-time polymerase chain reaction (RT-PCR), represent a clinical challenge. This study aimed to evaluate if the clinical manifestation at presentation, the laboratory and imaging results, and the prognosis of COVID-19 differ in patients who tested negative at the first RT-PCR compared with those who tested positive and also to evaluate if comorbid conditions patient-related or the period of arrival are associated with negative testing.

Study design: We retrospectively collected clinical data of patients who accessed the ED from March 1 to May 15, 2020.

Methods: We compared clinical variables, comorbid conditions, and clinical outcomes in the two groups by univariate analysis and logistic regression.

Results: Patients who tested negative at the first RT-PCR showed a higher prevalence of cardiopathy, immunosuppression, and diabetes, as well as a higher leukocyte and lower lymphocyte counts compared with patients who tested positive. A bilateral interstitial syndrome and a typical pattern at computed tomography scan were prevalent in the test-negative group. Test-negative patients were more likely to be admitted to the hospital but less likely to need admission in a high level of care ward. The false-negative rate increased from March to May.

Conclusion: False-negative RT-PCR COVID-19 patients present a similar spectrum of symptoms compared with positive cohort, but more comorbidities. Imaging helps to identify them. True positives had a higher risk of serious complications.

Introduction

After the COVID-19 outbreak, the broad clinical spectrum of symptoms and severity of this new syndrome has been widely described[1] in patients confirmed to have the disease by SARS-CoV-2 RNA detection by real-time polymerase chain reaction (RT-PCR) in upper airways swab.[2–4] At the very beginning of the pandemic, Xie et al. described the case of five "false-negative" patients[5] detected by chest computed tomography (CT). Thereafter, many other similar reports occurred,[6–9] mostly diagnosed by typical findings at chest CT.[6–9] The "threat" of false-negative worried many authors,[10,11] but the best diagnostic strategy is still controversial,[11–13] and the management of these cases raises some pragmatic questions about isolation, infection control, and prognosis.[10,11] The rate of patients presenting with COVID-19-like syndromes with a negative RT-PCR swab was estimated from 2% to 30%,[10–17] but this measure could be underestimated because of the lack of an alternative gold standard and because these subjects are often excluded from trials.[3] Technical preanalytical and analytical issues,[1,2,13–15] genetic diversity, and viral load kinetics at different

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anatomical sites[9,16,17] may cause a false-negative RT-PCR result. As a case in point, recently Baicry et al. investigated the first negative RT-PCR results of patients showing typical COVID-19 pneumonia at CT by different severity of illness and different viral load.[15]

In this study, we aimed to evaluate if the clinical manifestation at presentation, the laboratory and imaging results, and the prognosis of COVID-19 are different in patients diagnosed as COVID-19, who tested negative at the first RT-PCR, in comparison with those who tested positive. Furthermore, we evaluated if comorbid conditions patient-related or the period of arrival can influence the risk of negative testing at the first swab.

Methods

Turin province was the second most affected area in Italy during the "first wave" of the pandemic. All patients admitted at the emergency department (ED) of San Luigi Gonzaga University Hospital (Orbassano, Turin) with a suspected case of COVID-19 from March 1 to May 15, 2020, were retrospectively evaluated. Patients had consented to data collection for public health and research purposes at arrival, and the Institutional Ethics Committee approved the study (n° 5796, 15/04/2020).

Patients who tested positive for SARS-CoV-2 RT-PCR in nasal swabs were included. Patients who tested negative for SARS-CoV-2 RT-PCR at first nasal swab were included if admitted to an isolation room in COVID-19 wards or discharged at home in isolation with a clinical diagnosis of COVID-19 and fulfilled at least one epidemiological criteria or clinical criteria (Supplementary Table 1).

Test-negative COVID-19 patients were admitted to a special COVID ward area with isolation rooms to avoid the in-hospital spread of infection, and RT-PCR was repeated at 24 and 72 h after the first swab or a lower airways specimen was obtained. The presence of antibodies at a serological test after discharge was also recorded. After chart review by three experienced researchers, we excluded patients who were diagnosed otherwise at the end of the hospital stay.

For all the patients, we collected demographic data and comorbid conditions, symptoms at presentation, time from symptom onset, results of laboratory testing, and results of imaging.

We categorized radiologic findings according to literature classification[18–20] detailed in Supplementary Table 1.

The presence of an irregular pleural line at lung ultrasound or CT scan was also considered.

Outcomes

We collected data about respiratory support (continue positive airway pressure [CPAP], non-invasive mechanical ventilation, and invasive mechanical ventilation), admission or transfer to a higher level of care ward (intensive care unit [ICU] or high dependency unit [HDU]), length of stay in the higher intensity of care wards, in-hospital mortality, and total length of hospital stay.

Statistical analyses

Data were described as medians and interquartile ranges for quantitative variables and as absolute frequencies and percentages for categorical variables. Based on the non-normal distribution of the data assessed by the Shapiro–Wilk test, univariate comparisons were performed using the Wilcoxon rank-sum test in the group of positives to RT-PCR versus negative to RT-PCR. For categorical variables, we used the Chi-squared test or Fisher's exact test when the assumptions for conducting a chi-squared test were not met. Similarly, differences in outcomes (in-hospital mortality, ICU or HDU admission, need for respiratory support, and length of stay) were evaluated in the two groups. Moreover, multivariable logistic regression was performed to investigate confounding factors in the observed associations. Odds ratios (ORs) and 95% confidence interval (95% CI) of disease severity, comorbid conditions, and period of arrival on the result of the first RT-PCR were estimated, adjusted by age and sex. Finally, we run a sensitivity analysis in patients with moderate, severe, and critical COVID-19 (according to National Institutes of Health (NIH) classification[21]) to reduce possible biases because of the inclusion of asymptomatic patients and patients without pneumonia only in the test-positive group. All tests were two sided, and a P-value <0.05 was considered significant. Analyses were performed using R version 3.4.2.[22]

Results

Among patients who accessed the ED from March 1 to May 15, 2020, and consented to the study, 351 were tested with RT-PCR on a nasal swab. Two hundred and thirty-six patients (67%) tested positive, and 115 patients (32%) tested negative. All 115 met the inclusion criteria having either an epidemiological criterion or a clinical criterion and being admitted in COVID-19 ward (in isolation room) or discharged in isolation at home. Among the test-negative group, 49 of 115 patients were excluded after chart review by three experts because of a possible differential diagnosis that was found during the hospital stay, whereas 66 patients were confirmed to have COVID-19 at the end of the hospital stay (both by further testing and by clinical judgment). Among 66 patients who tested negative at the first RT-PCR, 16 patients (24%) tested positive at a further RT-PCR after a median of 11 (7–15) days, 21 (32%) patients tested persistently negative but showed the presence of antibodies, one did not repeat RT-PCR but showed the presence of antibodies, and 28 (42%) repeatedly tested negative at RT-PCR and were ruled in based on clinical diagnosis. None showed co-infection or the presence of alternative respiratory pathogens.

Two hundred and twenty-five patients overall (163 test-positive and 62 test-negative patients) were admitted to COVID-19 wards; 73 test-positive and four test-negative patients were discharged in isolation at home.

In this cohort, 86 patients overall reported to potentially have had a high-risk exposure to COVID-19 (16 were nursing home residents, 10 had close contact with a COVID-19 case, 41 were health workers, 18 reported a recent hospitalization or recurrently acceded outpatients' services [i.e. dialysis, oncology, day hospital, etc]). The presence of these epidemiological criteria was reported more frequently by test-negative patients than by test-positive patients. Clinical features of COVID-19 were present in most patients but were significantly higher in test-negative patients (65 [99%] and 212 [89%], respectively; $P = 0.023$). Symptom's prevalence was similar in both groups except for gastrointestinal symptoms more reported by test-positive patients (39 [16%] vs 4 [6%]; $P = 0.03$). Comparison of demographic data, epidemiological criteria, clinical data, and comorbid conditions in patients grouped by the first RT-PCR result is detailed in Table 1. Cardiac diseases, neurological chronic conditions, lung cancer, diabetes, and immunosuppression were more represented in the test-negative group than in the test-positive one. Patients who tested positive at a subsequent swab (n = 16) showed no significant differences in comorbid conditions compared with those who tested persistently negative (n = 50).

Laboratory tests at arrival showed that test-positive patients had a lower level of white blood cells (6.02 [4.59–8.13] vs 7.62 [5.12–11.50] $\times$ $10^3/\mu L$; $P = 0.02$) and higher lymphocyte count (1324.50 [1009.50–1789.50] vs 1120.00 [820.00–1710.00]/μL; $P = 0.013$), whereas lactate dehydrogenase levels were not

Table 1
Comparison of demographic data, epidemiological criteria, clinical data, and comorbid conditions in patients grouped by the first RT-PCR result.

Variable	Positive RT-PCR	Negative RT-PCR	*P*
N	236	66	
Age, median [IQR]	65.65 [52.57–79.10]	72.76 [55.41–81.32]	0.161#
Sex, n (%)			
Male	124 (52.5)	43 (65.2)	0.069°
Female	112 (47.5)	23 (34.8)	
Epidemiological criteria, n (%)			0.093°
No	175 (74.2)	42 (63.6)	
Yes	61 (25.8)	24 (36.4)	
Epidemiological criteria specification, n (%)			<0.001§
Contact to COVID	2 (3.3)	8 (33.3)	
Nursing home resident	8 (13.1)	8 (33.3)	
Repeated health care services users (dialysis, day hospital)	14 (23.0)	4 (16.7)	
Health worker	37 (60.7)	4 (16.7)	
Clinical criteria, n (%)			0.023§
No	24 (11.0)	1 (1.0)	
Yes	212 (89.0)	65 (99.0)	
Cough, dyspnea, n (%)			0.515°
No	62 (26.3)	20 (30.3)	
Yes	174 (73.7)	46 (69.7)	
Fever, n (%)			0.177°
No	59 (25.0)	22 (33.3)	
Yes	177 (75.0)	44 (66.7)	
Hyposmia, hypogeusia, n (%)			0.491§
No	227 (96.2)	62 (93.9)	
Yes	9 (3.8)	4 (6.1)	
Nausea, vomiting, diarrhea, n (%)			0.030§
No	197 (83.5)	62 (93.9)	
Yes	39 (16.5)	4 (6.1)	
Time from the onset of symptoms, median [IQR]	4.00 [2.00–7.00]	3.50 [2.00–8.00]	0.943#
Comorbidities			
Hypertension, n (%)			0.243°
No	130 (5.1)	31 (47.0)	
Yes	106 (44.9)	35 (53.0)	
Cardiopathy, n (%)			0.001°
No	197 (83.5)	43 (65.2)	
Yes	39 (16.5)	23 (34.8)	
Chronic obstructive pulmonary disease, n (%)			0.357°
No	202 (85.6)	60 (90.9)	
Yes	34 (14.4)	6 (9.1)	
Lung fibrosis, n (%)			0.390§
No	235 (99.6)	65 (98.5)	
Yes	1 (0.4)	1 (1.5)	
Lung cancer, n (%)			0.043§
No	233 (98.7)	62 (93.9)	
Yes	3 (1.3)	4 (6.1)	
Cancer, n (%)			0.121°
No	210 (89.0)	54 (81.8)	
Yes	26 (11.0)	12 (18.2)	
Immunosuppression, n (%)			<0.001°
No	227 (96.2)	52 (78.8)	
Yes	9 (3.8)	14 (21.2)	
Neurological disorders, n (%)			0.014°
No	202 (85.6)	48 (72.7)	
Yes	34 (14.4)	18 (27.3)	
Diabetes or other metabolic conditions, n (%)			0.001°
No	194 (82.2)	42 (63.6)	
Yes	42 (17.8)	24 (36.4)	
Renal failure, n (%)			0.711°
No	220 (93.2)	60 (90.9)	
Yes	16 (6.8)	6 (9.1)	

Data are described as absolute frequencies and percentages (in brackets) for categorical variables and as median and interquartile range [IQR] for quantitative variables. Comparisons are made with: #: Wilcoxon rank-sum test; °: Chi-squared test; §: Fisher's exact test.

significantly different in the two groups. The degree of respiratory failure assessed by oxygen partial pressure (PO_2), partial pressure of carbon dioxide (PCO_2), and the ratio PO_2/fraction of inspired oxygen (PO_2/FiO_2) at arrival was also similar.

Overall, 178 (75%) test-positive and 56 (84%) test-negative patients underwent lung ultrasound (LUS) at arrival; 210 (88%) test-positive and 61 (92%) test-negative patients had a chest X-Ray (CXR); CT was performed in 50 (21%) test-positive and 20 (30%) test-negative patients.

In Table 2, compared results of laboratory tests, imaging, and COVID-19 severity according to NIH classification[21] in test-positive and test-negative patients are reported. An LUS pattern of bilateral interstitial syndrome was prevalent in test-negative patients, followed by consolidation, whereas consolidation and unilateral

Table 2
Comparison of laboratory test, imaging results, and severity in patients grouped by the first RT-PCR result.

Variable	Positive RT-PCR	Negative RT-PCR	*P*
Laboratory test, median [IQR]			
PO_2 at arrival (mm Hg)	77.00 [60.00–94.75]	71.00 [61.00–82.00]	0.093#
PCO_2 at arrival (mm Hg)	34.50 [31.00–38.00]	35.00 [32.00–38.30]	0.33#
P/F at arrival	314.29 [235.71–392.86]	319.05 [250.00–369.05]	0.748#
Total WBC (K/μL)	6.02 [4.59–8.13]	7.62 [5.12–11.50]	0.002#
Lymphocytes (U/μL)	1324.50 [1009.50–1789.50]	1120.00 [820.00–1710.00]	0.013#
LDH (U/L)	330.50 [218.50–449.25]	288.50 [243.75–380.75]	0.526#
Imaging results, n (%)			
Chest X-ray (n = 271)			<0.001°
Pneumonia consolidation	40 (19.0)	15 (24.6)	
Interstitial syndrome	18 (8.6)	19 (31.1)	
Aspecific findings	87 (41.4)	15 (24.6)	
Normal CXR	65 (31.0)	12 (19.7)	
Lung ultrasound (n = 234)			<0.001§
Consolidation	54 (30.3)	13 (23.2)	
Monolateral interstitial syndrome	41 (23.0)	6 (10.7)	
Bilateral interstitial syndrome	34 (19.1)	26 (46.4)	
Pleural effusion	0 (0.0)	7 (12.5)	
Normal lung ultrasound	49 (27.5)	4 (7.1)	
CT scan (n = 70)			0.012§
Typical pattern	16 (80.0)	29 (58.0)	
Atypical pattern	0 (0.0)	5 (10.0)	
Undetermined	2 (10.0)	16 (32.0)	
Normal CT scan	2 (10.0)	0 (0.0)	
Irregular pleural line (n = 243)			0.751°
No	157 (84.0)	48 (85.7)	
Yes	30 (16.0)	8 (14.3)	
COVID-19 severity (NIH classification)			0.001§
Asymptomatic	16 (7)	0 (0)	
Mild illness	43 (18)	3 (5)	
Moderate illness	90 (38)	35 (53)	
Severe illness	70 (30)	26 (39)	
Critical illness	17 (7)	2 (3)	

Abbreviations: PO_2 = oxygen partial pressure; PCO_2 = partial pressure of carbon dioxide; P/F ratio = PO2/fraction of inspired oxygen (FiO2); wbc = white blood cells, LDH = lactate dehydrogenases, CT = computed tomography.
Data are described as absolute frequencies and percentages (in brackets) for categorical variables and as median and interquartile range [IQR] for quantitative variables. Comparisons are made with: #: Wilcoxon rank-sum test; °: Chi-squared test; §: Fisher's exact test.

interstitial syndrome prevailed in test-positive patients followed by bilateral interstitial syndrome. Interestingly, 27% of test-positive patients had a normal LUS pattern. Non-specific findings prevailed in test-positive patients at CXR, whereas interstitial syndrome was prevalent in test-negative patients. Eighty percent of test-positive patients had a typical CT scan compared with 58% of test-negative patients who showed a higher rate of undetermined and atypical findings ($P = 0.012$). In the test-positive group, we observed a prevalence of asymptomatic and mild cases by NIH COVID-19 severity classification. A moderate and severe illness prevailed in the test-negative group. The number of critical cases was significantly higher in the test-positive group. The sensitivity analysis in the subgroup of patients with moderate, severe, and critical illness is described in Supplementary Table 2: the comparison of the variables in this subgroup confirmed the findings obtained in the whole cohort.

Outcomes are described in Table 3. The need for respiratory support was similar in the two groups. We observed that test-negative patients were hospitalized mostly in general wards, and the admission in ICU or HDU was higher in test-positive patients, but mortality was similar.

Test-positive and test-negative patients showed up differently over the weeks ($P < 0.001$): more than half of the test-negative patients were admitted after April 1 (18 [27%] between April 1 and 15, 13 [20%] between April 15 and 30, and 9 [14%] at the beginning of May), whereas the majority of test-positive patients were admitted in March (30 [13%] between March 1 and 15 and 120 [51%] between March 16 and 31). No significant difference in severity was observed over time.

A logistic regression was performed, including sex, age, comorbid conditions, severity, and the period of arrival (see Fig. 1). Patients admitted to the ED at the end of April had 3.58 (1.10–12.76; $P = 0.040$) times the risk of testing negative at the first RT-PCR, those arriving in May had 7.66 (1.48–36.17; $P = 0.007$) times the risk of testing negative at the first RT-PCR, compared with those admitted at the beginning of march. Moreover, the risk of testing negative increases of 1.65 (1.24–2.24; $P = 0.001$) times with the increase of comorbid conditions.

Discussion

In this study, we aimed to characterize a subgroup of COVID-19 patients, who tested negative at the first RT-PCR and represent nearly 20% of our sample, in accordance with previous studies.[3,12,23] Interestingly, laboratory confirmation of the disease by further tests (microbiological and serological tests) was achieved only in nearly half of patients who tested negative at the first RT-PCR.

To define these cases, we used careful evaluation of clinical records of the entire hospital stay by three experienced physicians, which led to the exclusion of patients with a possible alternative diagnosis. This approach makes us confident of the inclusion in our cohort only of patients who were clinically COVID-19 and “false negative” at RT-PCR at the first swab. This comprehensive clinical approach was previously applied in case reports or case series only.[5,7,8,14,15,24–27,29,30] Observational studies, instead, usually have considered as false-negative patients only, those who turned positive afterward[12,16] or who showed a typical pattern at CT.[15]

Table 3
Comparison of respiratory support and outcomes in patients grouped by the first RT-PCR result.

Variable	Positive RT-PCR	Negative RT-PCR	*P*
CPAP/NIV, n (%)			0.540°
No	207 (87.7)	56 (84.8)	
Yes	29 (12.3)	10 (15.2)	
OTI during hospitalization, n (%)			1.000§
No	222 (94.1)	62 (93.9)	
Yes	14 (5.9)	4 (6.1)	
Emergency department outcome, n (%)			<0.001§
Discharged	73 (30.9)	4 (6.1)	
Admitted	157 (66.5)	62 (93.9)	
Transferred	3 (1.3)	0 (0.0)	
Death	3 (1.3)	0 (0.0)	
Hospital ward, n (%)			<0.001§
General ward	88 (55.0)	52 (83.9)	
High dependency unit	17 (10.6)	8 (12.9)	
Intensive care unit	55 (34.4)	2 (3.2)	
ICU/HDU LOS, median [IQR]	13.50 [8.00–23.00]	7.50 [4.50–21.25]	0.241#
Hospital LOS, median [IQR]	9.00 [0.00–20.00]	10.00 [6.00–20.50]	0.046#
Hospital outcome, n (%)			
Discharged			<0.001§
At home	57 (57.0)	37 (84.1)	
Rehabilitation	43 (43.0)	3 (6.8)	
Transferred to another hospital	0 (0.0)	4 (9.1)	
Death			0.307°
No	100 (63.7)	44 (71.0)	
Yes	57 (36.3)	18 (29.0)	

CPAP = continuous positive airway pressure; OTI = orotracheal intubation; ICU = intensive care unit; HDU = high dependency unit; LOS = length of stay.
Data are described as absolute frequencies and percentages (in brackets) for categorical variables and as median and interquartile range [IQR] for quantitative variables. Comparisons are made with: #: Wilcoxon rank-sum test; °: Chi-squared test; §: Fisher's exact test.

In our cohort, patients negative at the first RT-PCR had similar demographic characteristics and similar symptoms if compared with RT-PCR positive cases, except for gastroenteric symptoms. Test-positive patients showed more leukopenia and lymphocytosis, in agreement with Brendish et al.;[14] on the contrary, symptoms, the severity of respiratory impairment, and laboratory findings were similar in the test-positive and the test-negative group. A possible explanation may be found in the different designs and populations of the two studies: Brendish et al. included patients with respiratory symptoms with a differential diagnosis (a higher prevalence of smokers and chronic obstructive pulmonary disease), and they did not intend to compare false negative to RT-PCR cases with the confirmed COVID-19 cases.[14]

In our cohort, test-negative patients showed a higher prevalence of very typical patterns at LUS and CXR when compared with test-positive patients: this evidence remains when restricting the analysis to patients presenting with pneumonia (moderate, severe, or critical illness according to NIH classification[21]). We agree with other authors on the use of imaging to achieve a proper detection of cases[3,5–9] and acknowledge that having signs of interstitial pneumonia at LUS was prevalent in test-negative patients and could guide case definition. Similarly, we found that epidemiological factors in the history of false-negative patients were more represented.

When evaluating outcomes, corrected by severity, test-positive and test-negative patients showed a similar need for respiratory support and mortality rate. Nevertheless, the test-positive group showed a higher rate of admission in ICU/HDU, whereas most test-negative patients were admitted to general wards. We might hypothesize that test-positive patients had a higher viral load, which was seen to be related to more severe disease and a higher rate of detection by RT-PCR,[14,17] but this explanation needs further validation.

Regarding possible individual characteristics that could influence a false-negative result at RT-PCR, immunosuppression, as well as other comorbid conditions, such as cardiopathy and metabolic conditions, were associated with false-negative results at RT-PCR. To our knowledge, this is the first study to attempt to evaluate the effect of comorbid conditions on the possibility of having a negative swab. Other authors previously described merely case series[24,26] or evaluated possible effects of comorbid conditions on viral clearances.[27,29] The logistic regression confirmed that patients with more comorbid conditions had an increased risk of testing negative at the first RT-PCR.

The timing of the assay from symptom onset is known to influence the rate of RT-PCR positivity,[15,16,30] but in our cohort was similar among test-negative and test-positive patients. As Lippi et al.,[13] we assumed that technical issues in swab collection, transport, and analysis could have reduced the efficiency of swab tests in the first phase of the pandemic because of the overload of laboratories. On the contrary, we observed an increase in false negative in the late phase of the pandemic, which may be explained by a better clinical understanding of this illness.

Moreover, in the latter phase of pandemics in Piedmont, we admitted mainly older patients, often affected by multiple comorbid conditions,[28] who are at higher risk of testing negative. This trend is consistent with our findings that false-negative RT-PCR COVID-19 patients present more comorbidities compared with the positive cohort and, in line with a previous meta-analysis, that older age may affect RT-PCR sensitivity.[18,29]

As other authors,[2,24] we described RT-PCR-negative cases both during the first weeks of the first wave, when patients showed typical phenotypes, and later when we observed many atypical cases in older patients with a great burden of comorbid conditions.[24,25,28] The concurrence of atypical imaging patterns and clinical courses with negative microbiological results was challenging for the emergency physician in the later phase of the first Italian pandemic wave. We suggest that in patients with associated comorbid conditions, a comprehensive evaluation of laboratory results (leukopenia) and imaging (LUS and CT) may help to define cases that could be misdiagnosed by RT-PCR alone.

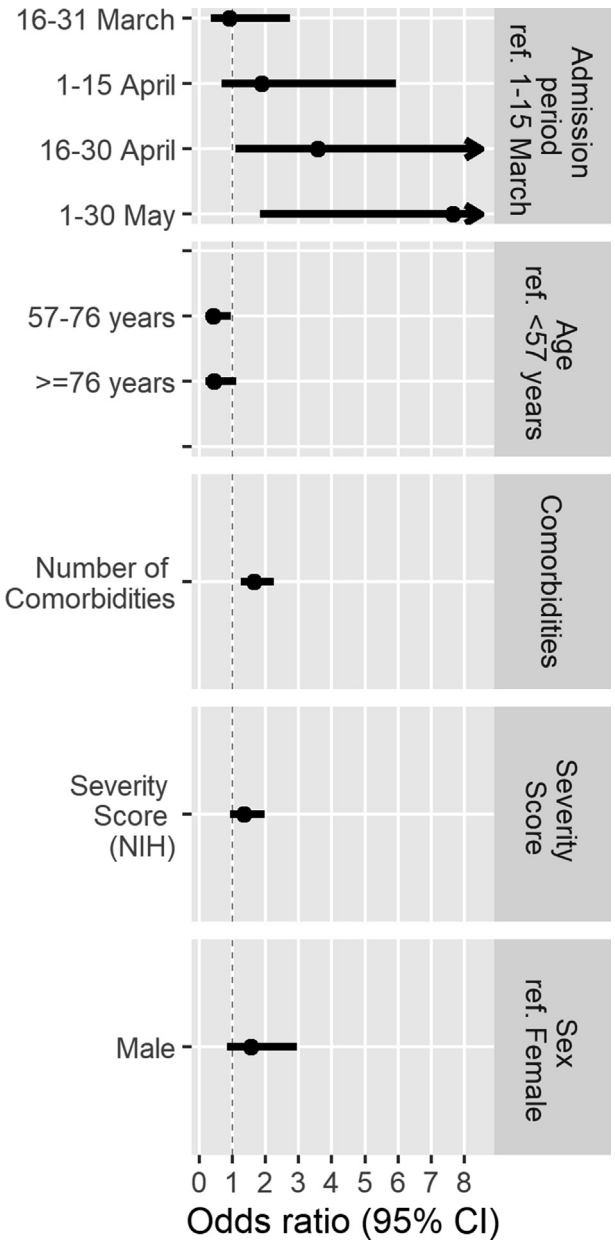

Fig. 1. Multivariable logistic regression for disease severity, comorbid conditions, and period of arrival on the result of the first RT-PCR, adjusted by age and sex. Odds ratios (ORs) and 95% confidence interval (95% CI) are shown.

Our findings must be interpreted bearing in mind that the data were collected at the very beginning of the pandemic crisis: in a similar context of uncertainties, both clinical experience and diagnostic performance of laboratory tests were rapidly evolving. In the following months, organizational and technological improvements have reduced many preanalytical and analytical issues reducing the rate of false-negative RT-PCR test. Thanks to the shared observations on false-negative cases, our colleagues were more confident in using lung ultrasound and in interpreting clinical, laboratory, and imaging findings, and in case of any discordant result, we implemented the diagnostic algorithm using antigenic testing, the evaluation of IgM antibodies, and the interpretation of cycle threshold value for RT-PCR to achieve diagnostic confirmation.

It would be of interest to confirm with further studies if the improvement in highly sensitive and specific tests or a combination of the newly available assays has reduced the incidence of "false-negative" at first RT-PCR during the subsequent pandemic waves.

Strengths and limitations

To the best of our knowledge, this is the first study to compare symptoms, comorbid conditions, and clinical variables in a broad cohort of COVID-19 cases with both positive and negative RT-PCR at the arrival in the ED. Moreover, it was previously unknown which possible modifier increases the risk of testing negative at RT-PCR. To avoid the comparison of two groups of patients at different stages of their clinical history of COVID-19 disease, we performed a sensitivity analysis restricted to moderate, severe, and critical COVID-19 cases confirming the results of the whole population.

This study has limitations: first, the single-center experience and the limited proportion of RT-PCR negatives compared with the other group. Then, its retrospective nature and the fact that serologic assays for SARS-CoV-2 were approved in Italy at the end of April limited the possibility of having our patients tested for SARS-CoV-2 antibodies. We tried to overcome the lack of laboratory confirmation of cases with a careful clinical evaluation by three experienced physicians to warrant an accurate group definition.

Author statements

Ethical approval

The Institutional Ethics Committee approved the study (n° 5796, 15/04/2020).

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None declared.

Competing interests

None declared.

Availability of data and material

Available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.puhe.2021.09.010.

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