ORIGINAL RESEARCH LETTER

False-negative RT-PCR in SARS-CoV-2 disease: experience from an Italian COVID-19 unit

To the Editor:

As of 25 May 2020, a total of 5 304 772 laboratory-confirmed cases and 342 029 deaths due to coronavirus diseases 2019 (COVID-19) have been reported worldwide [1], with the USA, Russia and Brazil being the most involved countries. In the absence of a specific treatment with established efficacy, and while waiting for the development of an effective and safe vaccine, prompt identification and immediate isolation of infected subjects still represent the most important countermeasures to stem the exponential rise in COVID-19 cases and related deaths.

To date, nucleic acid amplification testing such as real-time RT-PCR on respiratory specimens, particularly from naso- and oropharyngeal swabs, or nasopharyngeal wash or aspirate, represents the gold standard for the diagnosis of COVID-19 [2–4]. Nevertheless, more and more evidence is emerging regarding its lack of adequate sensitivity, questioning whether the current recommendations on COVID-19 diagnosis guarantee an adequate level of safety and effectiveness in the fight against the growing contagion.

On 23 March 2020, our respiratory ward was converted into a dedicated COVID-19 unit. Our hospital holds a total of four specialised COVID-19 units, including intensive and subintensive care units. As of 19 May 2020, 69 patients have been admitted to our unit with a diagnosis of COVID-19. Of these, 16 (23.2%) patients were admitted with high suspicion of COVID-19 based on clinical and chest high-resolution computed tomography (HRCT) findings, despite negative results of RT-PCR on two consecutive nasopharyngeal swabs at least.

Patients' characteristics are shown in table 1. Median delay between symptoms onset and arrival at the emergency department was 5 days (range 0–15 days). The most commonly reported symptoms were fever (87.5%), worsening dyspnoea (87.5%) and cough (43.7%); gastrointestinal symptoms were reported in two cases only. No recent close contacts with other subjects known to be infected by SARS-CoV-2 were reported. Most frequent comorbidities were arterial hypertension (68.7%) and type 2 diabetes mellitus (31.2%). Ongoing antihypertensive treatment with angiotensin convertin-enzyme inhibitors or angiotensin II receptor blockers was reported in eight cases.

Baseline arterial blood gas analysis revealed mild hypoxemia in 31.2% patients, whilst evidence of type 1 respiratory failure was found in 25% cases.

All patients underwent chest HRCT within 24 h of admission. 10 patients had a follow-up scan within a median interval time of 20 days (range 10–30 days). Typical findings of multilobar, peripherally distributed ground-glass opacities – in 10 (62.5%) cases associated with parenchymal consolidation – consistent with the suspicion of COVID-19 were found in all patients. Right upper and lower lobes were the most frequently involved areas.

According to local protocol, all patients were treated with azithromycin 500 mg every 24 h and hydroxychloroquine 200 mg every 12 h for 10 days, in addition to support therapy, which was established

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False-negative cases of #COVID19 are being increasingly reported. Laboratory diagnosis through RT-PCR testing alone lacks adequate sensitivity to be recommended as the only valid criterion to confirm COVID-19 diagnosis. https://bit.ly/2BLFnEe

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TABLE 1 Patients' characteristics

Age years	59.5 (30–86)
Males/females n	11/5
Comorbidities	
Arterial hypertension	11 (68.7%)
Type 2 diabetes mellitus	5 (31.2%)
Malignancies	2 (12.5%)
Pre-existing respiratory diseases	3 (18.7%)
Other	6 (37.5%)
Data at hospital admission	
Time between symptoms onset and admission days	5 (0–15)
Reported symptoms	
Fever ⩾38.0°C	14 (87.5%)
Dyspnoea	14 (87.5%)
Cough	7 (43.7%)
Gastrointestinal symptoms	2 (12.5%)
Other	3 (18.7%)
Body temperature °C	37.5 (37.2–38.9)
Blood gas analysis[#]	
P_{aO_2} mmHg	69 (48–98)
P_{aCO_2} mmHg	31 (27–38)
pH	7.46 (7.39–7.52)
P_{aO_2}/F_{IO_2} mmHg	329 (228–467)
Laboratory findings	
Haemoglobin g·dL^{-1}	13.8 (9.2–15.0)
Red blood cells ×10^{12} per L	4.7 (3–0–5.4)
White blood cells ×10^{9} per L	8.6 (3.8–20.6)
Neutrophils ×10^{9} per L	7.2 (3.9–18.9)
Lymphocytes ×10^{9} per L	0.8 (0.4–2.0)
Monocytes ×10^{9} per L	0.4 (0.1–3.0)
Platelets ×10^{9} per L	231.0 (102.0–561.0)
C-reactive protein g·dL^{-1}	13.6 (1.1–37.5)
Ferritin µg·L^{-1}	371 (80–1364)
D-dimer µg·L^{-1}	1256 (197–4473)
Respiratory support	
Oxygen	12 (75.0%)
Positive end-expiratory pressure	7 (43.7%)
High-flow nasal cannula	1 (6.2%)
Noninvasive ventilation	0 (0%)
Orotracheal intubation	0 (0%)
Length of hospitalisation days	25 (8–49)
Outcome	
Discharge home	14 (87.5%)
Intensive care unit	0 (0%)
Death	2 (12.5%)
RT-PCR assays	
Nasopharyngeal swabs	66 (89.2%)
BALF	6 (8.1%)
Rectal swabs	2 (2.7%)
RT-PCR repetitions during hospital stay/time from symptom onset days	
3	16 (100%)/8 (2–20)
4	13 (81.2%)/18 (9–27)
5	9 (56.2%)/21 (10–27)
6	4 (25.0%)/24 (17–24)
RT-PCR positive results[¶]	
Patients	3 (18.7%)
Testing	3 (4.1%)
Anti-SARS-CoV-2 serum antibodies[+]	
IgM	8 (88.9%)
IgG	9 (100%)
Time from symptom onset days	25 (20–35)

Data are presented as median (range) or n (%), unless otherwise stated. F_{IO_2}: inspiratory oxygen fraction; P_{aO_2}: arterial oxygen tension; P_{aCO_2}: arterial carbon dioxide tension; BALF: bronchoalveolar lavage fluid. [#]: F_{IO_2} 0.21; [¶]: after two first negative results; [+]: N=9.

on an individual basis. No antiretroviral therapy was administered. Median length of hospitalisation was 25 days (range 8–49 days). Major complications (*i.e.* deep venous thrombosis, pulmonary embolism and acute kidney injury) were reported in four cases. Exitus occurred in two extremely frail patients due to nonresponsive respiratory failure.

A total of 74 RT-PCR assays were performed (median 5 per patient): 66 (89.2%) nasopharyngeal swabs, six (8.1%) bronchoalveolar lavage fluids (BALF) and two (2.7%) rectal swabs. As shown in table 1, each patient had a minimum of three RT-PCR assays. Only three (4.0%) assays were positive (median time to first positive sample 9 days from symptoms onset).

Of note, nine (56.2%) patients were also tested for anti-SARS-CoV-2 serum antibodies at a median time of 17 days (range 14–25 days) from hospitalisation and 25 days (range 20–35 days) from symptoms onset, all of them being positive for IgG antibodies and eight out of nine for IgM antibodies too. The only IgM-undetermined case had the serology testing performed after 14 days from hospital admission, corresponding to 25 days after symptom onset. For the other seven patients, serology testing was not available yet at the time of their discharge.

Our experience follows the growing number of published papers concerning several cases of RT-PCR-negative COVID-19 patients. Anecdotal cases have been first reported [5–9]. Interestingly, Li *et al.* [7] briefly mention a local incidence of false-negative COVID-19 patients of 20% within their hospital, very similar to what it was observed in our unit.

The leading role played by chest CT scan in the identification and management of false-negative patients with COVID-19 has been highlighted by multiple study groups. Fang *et al.* [10] retrospectively studied 51 patients affected by COVID-19 who underwent RT-PCR and chest CT scan: 70.6% had initial RT-PCR assay positive for SARS-CoV-2; 29.4% needed a second assay at least to test positive. However, CT scan performed within 3 days from first swab was found to have a detection rate of 98.0%. Using the first RT-PCR assay as a reference, Long *et al.* [11] found a similar sensitivity for chest CT scan (97.2%) in a retrospective analysis involving 36 patients; on the contrary, 16.7% cases would have been missed if RT-PCR was not repeated at least twice.

In a retrospective cohort of 1014 Chinese patients, Ai *et al.* [12] found detection rates for throat swab and CT scan of 59% and 88%, respectively. Taking RT-PCR result as a reference, chest CT showed sensitivity of 96.5%, specificity of 25.4%, positive predictive value of 65.3% and negative predictive value of 83.3%. CT performance seemed to be even slightly better among older patients (*i.e.* >60 years) and females.

An even lower sensitivity of RT-PCR testing was shown by Li *et al.* [13] in their retrospective analysis of 610 patients from Wuhan city with clinically and radiologically combined confirmation of COVID-19 diagnosis. In their cohort, only 39.5% cases had at least one positive RT-PCR result.

As for every laboratory test, real-time RT-PCR has intrinsic limitations that might significantly affect its accuracy in the diagnosis of COVID-19. False-negative results may depend on several pre-analytical and analytical vulnerabilities, such as inadequate procedures for specimen collection, handling, transport and storage; collection of inadequate material (quality or volume); sample contamination; execution of the test outside of the diagnostic window; use of nonvalidated assays; and many others [14].

The combination of RT-PCR analytical vulnerability and major uncertainties about SARS-CoV-2 infection kinetics make it extremely difficult to accurately define the diagnostic window for the test itself, which becomes even harder to estimate on an individual basis rather than from an epidemiological point of view. Moreover, development of recombinant forms of SARS-CoV-2 may adversely affect the diagnostic accuracy of nucleic acid-based tests.

The source of respiratory specimen to be tested represents another critical issue. Collecting specimens at the right time from the right anatomic site seems crucial for laboratory diagnosis of SARS-CoV-2 infection through RT-PCR. Wang *et al.* [15] recently examined 1070 specimens collected from 205 patients with COVID-19. In their study, BALF guaranteed the highest positive rate (14 out of 15, 93.3%), followed by sputum (75 out of 104, 72.1%), nasal swabs (five out of eight, 62.5%), fibrobronchoscope brush biopsy (six out of 13, 46.1%), pharyngeal swabs (126 out of 398, 31.7%), faeces (44 out of 153, 28.8%) and blood (three out of 307, 1.0%). None of the 72 urine specimens tested positive. Similar results were found in the analysis on 866 respiratory samples performed by Yang *et al.* [16], who also analysed the impact of time from symptom onset and disease severity on the detection rate of RT-PCR assays.

These data strongly encourage us to manage patients with a high pre-clinical likelihood (as everyone should be considered in those countries with high level of contagion) and typical clinical and radiological features as affected by COVID-19, independently of the result of real-time RT-PCR, especially if performed on specimens collected from the upper airways. Acquisition of lower respiratory tract samples

should be always considered in the event of one or more negative RT-PCR assays, particularly in those with severe disease, where BALF and sputum provide the highest positive rates. If COVID-19 is suspected, HRCT scan should be always performed at hospital admission, together with or even before swabs, as this has been shown to correctly lead the clinical management yet from the earliest stages of disease and to provide the highest detection rates after very short time from symptoms onset.

Further studies to better assess the kinetics of SARS-CoV-2 infection in human airways and the associations between viral load, likelihood of viral localisation and symptoms are strongly encouraged to increase the analytical sensitivity of RT-PCR testing.

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