Negative nasopharyngeal swabs in COVID-19 pneumonia: the experience of an Italian Emergency Department (Piacenza) during the first month of the Italian epidemic

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Summary. Coronavirus disease (COVID-19) is a systemic disease which can cause multiple organ failure and death primarily due to vascular endothelium injury (1). Severe acute respiratory distress syndrome (ARDS) is the main cause of death: its management and treatment should be tailored to the individual COVID-19 patient’s phenotype (2). In Italy on 31st March 2020, 113,312 of COVID-19 cases have been registered, among them 14,324 have had fatal outcomes according to the Italian National Institute of Health surveillance system. Early diagnosis of COVID-19 is paramount for disease treatment and infection control. Naso-pharyngeal (NP) swab is commonly used as screening and diagnostic tool for COVID-19, but in some cases it can be resulted negative even in presence of clinical and epidemiological criteria, and typical radiological and laboratory findings of COVID-19, as we have observed. Here we report our experience in the first month of the Italian epidemic. We strongly recommend clinicians to maintain a high index of suspicion for COVID-19, regardless of the persistence negativity of NP swabs, and not to delay the initiation of therapy in presence of typical clinical, radiological and laboratory findings of COVID-19. (www.actabiomedica.it)

Key words: COVID-19, nasopharyngeal swab, RT-PCR, serology, Italian epidemic

Coronavirus disease (COVID-19) is a systemic disease which can cause multiple organ failure and death primarily due to vascular endothelium injury (1). Severe acute respiratory distress syndrome (ARDS) is the main cause of death: its management and treatment should be tailored to the individual COVID-19 patient’s phenotype (2). In Italy on 31\textsuperscript{st} March 2020, 113,312 of COVID-19 cases have been registered, among them 14,324 have had fatal outcomes according to the Italian National Institute of Health surveillance system. Early diagnosis of COVID-19 is paramount for disease treatment and infection control. In absence of specific treatments and vaccines, the national lock-down has been paramount for containing the spread of SARS-CoV-2.

From 6\textsuperscript{th} to 23\textsuperscript{rd} March 2020, 1,569 patients have been admitted to the Emergency Department (ED) of “Guglielmo da Saliceto Hospital” in Piacenza (Emilia-Romagna, Northern Italy) for acute respiratory failure during COVID-19 outbreak. All the patients were tested for COVID-19 infection by nasopharyngeal (NP) swab. Among all the specimens sent to the Laboratory for real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR), 29 specimens (0.02\%) resulted negative for SARS-CoV-2 (3). The low percentage of negative NP swabs is probably due to the correct way to collect samples, which is of paramount importance to minimize the false negative rate among

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COVID-19 positive patients (4). Sampling procedure is the most important factor in ensuring accurate results. NP swab is the easiest and preferred method to collect specimens, but it is extremely important to properly perform the procedure reaching the posterior rhinopharyngeal tonsil region. Health care workers need to be correctly trained to minimize false negative results (5, 6).

All the patients (2 females, 27 males, mean age 69 +/- 12 years, range 45 - 91) complained of dyspnoea and fever in the last 10 days (mean time 10 +/- 6 days). None of them had a history of neoplasia or pulmonary diseases, including COPD. Nineteen patients needed high flux oxygen therapy. Nine patients were treated with continuous positive airway pressure (CPAP): among them, 5 patients were subsequently intubated and 2 died of ARDS. Only one patient was intubated at admission and immediately transferred in the Intensive Care Unit; he died after 16 days of hospitalization. In all the cases antiviral therapy with Darunavir/Cobicistat, hydroxychloroquine, intravenous corticosteroids and low molecular weight heparin were promptly started.

Point-of care lung ultrasound was performed at admission in 22/26 (85%) patients and showed thickened pleural line, B lines (focal in mild infection, multifocal and confluent in advanced stage and critically ill patients), small subpleural consolidations with or without air bronchograms (7). Chest CT scan demonstrated typical radiographic features in 26/29 patients, including ground glass opacities, crazy-paving pattern and patchy consolidation (8). Three patients were investigated with chest X-Ray, which confirmed pneumonia multiple bilateral multifocal infiltrations.

As recently reported, CT scan can detect COVID-19 pneumonia in patients with initial negative RT-PCR results (9-10), and 60% to 93% of investigated patients have initial positive chest CT consistent with COVID-19 before the initial positive RT-PCR results (11).

In presence of high clinical suspicion for acute respiratory failure and radiographic findings consistent with COVID-19 pneumonia, we repeated the NP swabs 48-72 hours after the initial RT-PCR test: 17/29 (58%) resulted again negative. Mycoplasma, Legionella and Streptococcus pneumoniae on blood and urine samples were ruled out in 7/17 patients (not tested in 10 patients). Blood cultures and procalcitonin resulted negative in all the patients, excluding bacterial infections.

Nine out 17 patients had multiple consecutive negative NP swabs (3-4 specimens). Among them, 3 patients were intubated after an unsuccessful attempt of non invasive ventilation: one patient was a 69 year-old male with a history of hypertension, admitted to the ED for fever and shortness of breath since the last 4 days; the second one was a 65 year-old man with an unremarkable past medical history, who complained at admission fever and progressive dyspnoea in the last 6 days. In both the cases bronchoaspirate resulted positive for Pseudomonas aeruginosa and ceftepide was started. Bronchoalveolar lavage (BAL) was not performed. The first case died after 10 days of intubation. At time of writing, the second patient is tracheostomized and still hospitalized, but his overall conditions are slowly improving. The third case was a 55 year-old man in good health who complained for fever and cough over the previous 8 days. He had a moderate acute respiratory failure (PaO2/FiO2 280 mmhg) initially treated with continuous positive airway pressure (CPAP) for 5 days and then intubated for the worsening of respiratory failure and the increase of ground glass opacities and the detection of newly multiple bilateral consolidations at chest CT scan. Multiple bronchoaspirates were all negative. Repeated procalcitonin was still in the normal range. After 2 weeks, he was extubated, and after 3 weeks he was discharged with no need of oxygen therapy at home. The remaining 8 patients with 3 or more negative NP swabs were discharged in good health after complete resolution of respiratory failure and no need of oxygen.

Our experience highlights the importance to keep an open mind if there is a high clinical suspicion of COVID-19 even with multiple negative NP swabs. NP swab can result as false negative for several causes, including: the sampling technique and transportation process (12). In a recent review by Venter and Richter (13) RT-PCR is the recommended test for diagnoses of acute cases, while serological assays are useful to define epidemiological issues, such as attack rate in the population, and to identify immune individuals, since IgM and IgG antibody responses are only detectable
after approximately 6–15 days post-disease onset. Sputum or BAL can be used as alternative specimen for the diagnosis of COVID-19 (14-16). In patients with ARDS and need of intubation, sputum specimen can be collected during the intubation procedure, or BAL should be performed after intubation (17, 18). In our experience, COVID-19 patients with ARDS treated with CPAP/NIV can present severe hypoxemia without dyspnea as recently reported by Gattinoni et al. (2). In this context, patient’s compliance to bronchoscopy and BAL could be reduced, and the increased risk of coronavirus transmission due to close contact with respiratory droplets and aerosolization from the procedure should be taken in account.

Based on our experience and the current literature, we strongly recommend that clinicians maintain a high index of suspicion for COVID-19, regardless of the persistence negativity of NP swabs, and not to delay the initiation of therapy in presence of typical clinical, radiological and laboratory findings of COVID-19.

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