Acta Biomed 2020; Vol. 91, N. 3: e2020024 DOI: 10.23750/abm.v91i3.9979

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

Erika Poggiali[1], Andrea Vercelli[1*], Giovanni Battista Vadacca[2], Roberta Schiavo[3], Silvana Mazzoni[1], Eva Ioannilli[1], Elena Demichele[1], Andrea Magnacavallo[1]*

[1] Emergency Department, "Guglielmo da Saliceto" Hospital, Piacenza, Italy; [2] Biochemestry Unit, Clinical Pathology Department, "Guglielmo da Saliceto" Hospital, Piacenza, Italy; [3] Microbiology Unit, Clinical Pathology Department, "Guglielmo da Saliceto" Hospital, Piacenza, Italy

Summary. Coronavirus disease (COVID-19) is a systemic disease which can cause multiple organ failure and death primarly due to vascular endothelium injury. 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. 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 primarly 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, 113312 of COVID-19 cases have been registred, 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 lockdown has been paramount for containing the spread of SARS-CoV-2.

From 6th to 23rd March 2020, 1569 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

*The authors equally contributed to this work.

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 adimission 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 bilteral 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 bacteral infections.

Nine out 17 patients had multiple consecutive negative NP swabs (3-4 specimens). Among them, 3 patients were intubated after an unsuccessfull 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 ceftazidime 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 continous 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.

Acknowledgements

The authors wish to thank all the staff of the "Guglielmo da Saliceto" Hospital for their help, energy, resiliency and flexibility in such a difficult and hard global health crisis.

Authors' contributions: All listed authors meet the criteria for authorship and have contributed to the manuscript writing and reviewing.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Grasselli G, et al, COVID-19 Lombardy ICU Network. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. JAMA 2020. Published online April 6, 2020. doi: 10.1001/jama.2020.5394.
2. Gattinoni L, Chiumello D, Caironi P, Busana M, Romitti F, Brazzi L et al. COVID-19 pneumonia: different respiratory treatments for different phenotypes? Intensive Care Med 2020. [Epub ahead of print].
3. World Health Organization. Novel Coronavirus (2019-nCoV) technical guidance: Laboratory testing for 2019-nCoV in humans. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance
4. Petruzzi G, De Virgilio A, Pichi B, Mazzola F, Zocchi J, Mercante G, et al. COVID-19: Nasal and Oropharyngeal Swab. Head Neck 2020. doi: 10.1002/hed.26212. Epub 2020 Apr 30.
5. Marty FM, Chen K, Verrill KA. How to obtain a nasopharyngeal swab specimen. N Engl J Med 2020;382:e76-e76.
6. Patel ZM. Reflections and new developments within the COVID-19 pandemic. Int Forum Allergy Rhinol 2020;382:587-588.
7. Poggiali E, Dacrema A, Bastoni D, Tinelli V, Demichele E, Mateo Ramos P, et al. Can lung US help critical care clinicians in the early diagnosis of novel coronavirus (COVID-19) pneumonia? Radiology 2020. doi: 10.1148/radiology.2020200847
8. Chung M, Bernheim A, Mei X, Zhang N, Huang M, Zeng X, et al. CT imaging features of 2019 novel coronavirus (2019-nCoV). Radiology 2020. doi: 10.1148/radiol.20202000230
9. Huang P, Liu T, Huang L, Liu H, Lei M, Xu W, et al. Use of chest CT in combination of RT-PCR assay for the 2019 novel coronavirus but high clinical suspicion. Radiology 2020. doi: 10.1148/radiol.2020200330.
10. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for typical 2019-nCoV pneumonia: relationship to negative RT-PCR testing. Radiology 2020. doi: 10.1148/radiol.2020200343
11. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology 2020; Feb 26:200642. doi: 10.1148/radiol.2020200642
12. Tang YW, Schmitz JE, Persing DH, Stratton CW. The Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges. J Clin Microbiol 2020. doi: 10.1128/JCM.00512-20. [Epub ahead of print]
13. Venter M, Richter K. Towards effective diagnostic assays for COVID-19: a review. J Clin Pathol 2020; May 13; jclinpath-2020-206685. doi: 10.1136/jclinpath-2020-206685. Online ahead of print.
14. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 2020; 20 (4): 411-412. doi: 10.1016/S1473-3099(20)30113-4. Epub 2020 Feb 24.
15. Lippi G, Mattiuzzi C, Bovo C, Plebani M. Current laboratory diagnostics of coronavirus disease 2019 (COVID-19). Acta Biomed 2020; 91(2):137-145. doi: 10.23750/abm.v91i2.9548
16. Pasomsub E, Watcharananan SP, Boonyawat K, Janchompoo P, Wongtabtim G, Suksuwan W, et al. Saliva sample as a non-invasive specimen for the diagnosis of coronavirus

disease-2019 (COVID-19): a cross-sectional study. Clin Microbiol Infect 2020 [Epub ahead of print]
17. Torrego A, Pajares V, Fernández-Arias C, Vera P, Mancebo J. Bronchoscopy in COVID-19 Patients with Invasive Mechanical Ventilation: A Center Experience. Am J Respir Crit Care Med.2020. [Epub ahead of print].
18. Wahidi MM, Shojaee S, Lamb CR, Ost D, Maldonado F, Eapen G, Caroff DA, et al. The Use of Bronchoscopy During the COVID-19 Pandemic: CHEST/AABIP Guideline and Expert Panel Report. Chest 2020. pii: S0012-3692(20)30850-3. doi: 10.1016/j.chest.2020.04.036

Received: 1 June 2020
Accepted: 16 June 2020
Correspondence:
Poggiali Erika, M.D.
Emergency Department, "Guglielmo da Saliceto" Hospital,
Via Giuseppe Taverna 49 - 29121, Piacenza, Italy
E-mail: poggiali.erika@gmail.com; E.Poggiali@ausl.pc.it