**An electronic nose for COVID-19 detection by means of exhaled breath analysis**

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**1. Introduction**

The COVID-19 has revolutionized our lives, and imposed extraordinary and new challenges on the scientific community worldwide 1. In this context, chemical engineering has offered methods and technologies to face with the emergency. More in detail, chemical engineers around the word have focused on various aspects associated to the COVID-19 pandemic, such as the study of the nature and physiological mechanisms of the virus 2, transport mechanisms in the atmosphere 3, virus stability on materials (e.g., plastic, steel, glass, paper) constituting everyday objects, the production and testing of face masks 4, and the research on vaccines, drugs and novel chemicals or technologies for disinfections 5-7.

Many efforts were also devoted to diagnosis. The SARS-CoV-2 can cause fever, cough and even respiratory failure, which is a severe condition that requires prompt intervention 8. Nevertheless, those symptoms are not specific for SARS-CoV-2 but can be also related to other infections caused by other viruses or bacteria. For this reason, the research in this filed aimed at investigating potentialities and drawbacks of different diagnostic tests (e.g., reverse transcription-polymerase chain reaction (RT-PCR), serological test and antigenic test) or developing alternative approaches, is particularly active.

The analysis of endogenous Volatile Organic Compounds (VOC) of exhaled breath represents an interesting tool to early identify COVID-19 and improve personalizing treatments. Those VOC, resulting from metabolic processes potentially altered by the presence of the disease, can, in fact, provide information on the conditions of subjects, and guide the choice of treatment 9.

Electronic noses (ENs), instruments designed for mimicking human olfaction by means of the combination of an array of non-specific gas sensors with machine learning tools 10, could be considered as a novel, non-invasive and fast diagnostic technique for the rapid identification of COVID-19 patients 9.

Several research studies proved the applicability of this technology to exhaled breath analysis to support non-invasive diagnosis of various diseases 11-14, and recent preliminary studies also evaluated the use of ENs for diagnosing SARS-CoV-2 infection 15-16.

Even though exhaled breath analysis by EN might enable identifying treatable traits in patients with different respiratory disorders, its application to patients with respiratory failure, as it is the case of COVID-19 patients, remains a challenge. Diagnostic systems for the study of VOCs in the exhaled breath should filter out or compensate for ambient VOCs altering the composition of exhaled breath and affecting the diagnosis. In general, systems proposed in the scientific literature obtain lung wash-out by making the subject inhale through a charcoal filter, which adds respiratory load 17. Therefore, this approach is not suitable for respiratory failure patients.

The present study proposes an experimental set-up for exhaled breath sampling, specifically designed for patients with acute respiratory failure. The set-up was tested with a feasibility study carried out at the hospital ASST Papa Giovanni XXIII, Bergamo, Italy, involving 1) patients with COVID-19 respiratory failure, 2) asymptomatic patients with SARS-CoV-2 infection and 3) controls.

**2. Materials and Methods**

**2.1. Set-up for exhaled breath collection**

The experimental set-up comprises a T-piece to allow a bias flow through the breathing circuit, a non-rebreathing valve (BB089YBPV, Burke & Burke Spa, Milan, Italy), and an antiviral filter to avoid patients cross-contamination (Figure 1). The system is connected to the hospital medical gasses pipeline system to provide clean gasses to prevent sample contamination due to ambient air VOCs influencing the composition of exhaled breath and potentially affecting the results. The connection to the medical gasses pipeline system also allows regulating, according to the patient's needs, the fraction of inspired oxygen (FiO2). The FiO2 is an estimation of the oxygen content a person inhales and it is, thus, involved in gas exchange at the alveolar level.

The bias flow of medical gasses passing through the breathing circuit and the FiO2 can be adjusted using two rotameters on the air and oxygen lines. In the present study, we used a bias flow of 45 L/min and a FiO2 of 0.21. A 10 cm-long tube at the outlet of the T-piece guarantees that the subject inhales only the gas from the pipeline system and not the environment air.

A Nalophan bag with a capacity of 5L was connected to the expiratory outlet of the non-rebreathing valve to collect exhaled gas.



**Figure 1.** Proposed breath sampling apparatus a) wash-out phase b) exhaled breath sampling.

**2.2. Population**

The feasibility study carried out at the hospital ASST Papa Giovanni XXIII, Bergamo, Italy (ethical committee approval number 223/20) involved 55 subjects: 1) 25 patients with respiratory failure positive to SARS-CoV-2; 2) 8 asymptomatic subjects positive to SARS-CoV-2; 3) 22 healthy controls. Table 1 summarizes the characteristics of study participants.

Infection with SARS-CoV-2 was determined by PCR test on oropharyngeal swabs. Controls were age-matched subjects with a negative molecular swab to SARS-CoV-2 without chronic respiratory disorders who did not present any respiratory tract infection in the last 90 days.

**Table 1.** Characteristics of study participants.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **SARS-CoV-2 and respiratory failure** | **SARS-CoV-2 and asymptomatic** | **Controls** | **p-value** |
| N | 25 | 8 | 22 |  |
| Male sex, n | 17  | 3  | 14  | 0.295 |
| Age (years), median [IQR] | 50 [18.46] | 50 [24.15] | 50 [23.76] | 0.403 |
| Smokers, n  | 2  | 2 | 1  | 0.219 |
| Ex-smokers, n | 5  | 2  | 5  | 0.948 |
| Subjects with other comorbidities, n | 10  | 5  | 3  | 0.425 |

**2.3. Experimental protocol**

Subjects were asked not to eat, drink, or smoke in the two hours before the study. The sampling protocol consisted of two phases:

1. Wash out phase: The subject breathed for 3 minutes through the set-up with the exhalation line of the non-rebreathing valve open, which allowed inhalation of a mixture of medical gasses from the hospital pipeline system and exhalation into the ambient. The subject wore a nose clip to avoid nasal respiration and breathed through a mouthpiece.
2. Exhaled breath sampling: At the end of the wash-out phase, patients hold their breath while the operator connects a Nalophan bag to the expiratory line of the non-rebreathing valve to collect exhaled gas. Approximately five breaths were sampled, and the whole procedure lasted less than 5 min.

The bags with exhaled breath samples were stored for 2 to 24 hours after collection in the same room where the EN was installed to reduce the moisture content of samples 18. This sample conditioning phase, exploiting the Nalophan permeability to humidity, allowed preventing the potential interference of moisture on EN responses, thereby ensuring good stability and reproducibility of the measurements 19[9].

After conditioning, exhaled breath samples were analysed by a commercial e-nose (EOS-AROMA, SACMI s.c) equipped with 4 Metal Oxide (MOX) Sensors. The sampled exhaled gas were sucked at a constant flow rate of 50 mL/min for 20 min into the sensor chamber using a vacuum pump. Then, room reference air was sucked into the sensor chamber at 50 mL/min for 20 min to restore the sensors' baseline. Each sample was analyzed once.

**2.4. Data processing**

The data processing procedure developed for this study comprises three steps: features extraction, features selection, and pattern recognition. A total of 112 features representative of both steady-state and transient conditions were extracted from sensor time responses, as reported by 19. Then, a feature selection model based on Boruta algorithm 20 was implemented to identify the features that better discriminated between respiratory failure patients with SARS-CoV-2 and controls. Selected features were processed by Principal Component Analysis (PCA) 20[13], and PCA scores were used as inputs of a Support Vector Machine (SVM) classifier 21.

A 10-fold cross-validation approach was used to optimize features selection and pattern recognition and estimate the classification performances of the proposed model. The diagnostic accuracy of the model was evaluated using the Area Under the Receiving Operating Characteristic (ROC) 22 and expressed both as Area Under the ROC curve (AUC), sensitivity, and specificity.

**3. Results and discussion**

The breath sampling set-up proved suitable clinical setting and well tolerated by the patients. All measurements were completed successfully.

Concerning the EN capability to differentiate odour fingerprints of breath samples from respiratory failure patients with SARS-CoV-2 and controls, Figure 2 illustrates the score plot of the PCA built on the training dataset. Respiratory failure patients with SARS-CoV-2 and controls clustered in different plot regions: samples from controls clustered in the upper right part of the plot, while samples from respiratory failure patients with SARS-CoV-2 clustered in the lower left portion. This evidence suggests that EN has the potential to identify the specific odour fingerprint associated with respiratory failure due to COVID-19.

We superimposed data from asymptomatic SARS-CoV-2 patients as an exploratory analysis on the PCA model. Interestingly, the asymptomatic SARS-CoV-2 infected subjects fell between the two clusters, i.e., respiratory failure patients with COVID-19 and controls. This result, also confirmed by the analysis of the 2-Norm of PCA scores (Figure 3), suggested that the EN may provide information about the presence of the infection and the severity of the disease.



**Figure 2.** Principal components of selected features in asymptomatic SARS-CoV-2 infected patients (red circles), respiratory failure patients with COVID-19 (blue circles), and control subjects (green circles).



**Figure 3.** 2-Norm of scores of all the Principal Component of COVID-19 patients with respiratory failure, asymptomatic SARS-CoV-2 infected patients, and healthy controls. The boundaries of the boxes indicate the 25th and 75th percentiles, the lines within the boxes mark the median values. Whiskers indicate the 90th and 10th percentiles. Closed circles are considered outliers.

The classification model achieved a sensitivity of 92% (CI95% 87 - 99), a specificity of 68% (CI95% 54 - 78), and an AUC of 81% (CI95% 54 – 78).



**Figure 8.** Receiving Operating Characteristic (ROC) curve.

**4. Conclusions**

The feasibility study here described proved the potentialities of EN technologies for the exhaled breath analysis aimed at diagnostic purposes. It can provide an alternative to traditional clinical tests to ease the identification of respiratory failure causes' and improve patients' management. However, this type of analysis in patients with respiratory failure is challenging because of patients' unstable conditions and VOCs containats in the environment that can affect the EN analysis (e.g., VOCs associated with cleaning chemicals, drugs, other diseases, etc).

This study introduces an innovative breath sampling system specifically designed for respiratory failure patients, overcoming the issues associated with using charcoal filters to compensate for ambient VOCs, which add respiratory load and are usually not certified to be used with oxygen-enriched mixtures. The proposed set-up uses medical gasses available from the hospital pipeline system to wash out the lungs from ambient VOCs and collect uncontaminated exhaled breath without increasing the patient's work of breathing. Such a solution also allows the investigator to provide oxygen-enriched air with fine control of the FiO2, as commonly required by patients with respiratory failure.

This system was evaluated on stable patients with and without respiratory failure due to SARS-CoV-2 infection and on healthy controls and proved feasible for the critical care setting. At first, a classification model to investigate whether the device can detect differences in the odour fingerprints of patients with respiratory failure compared with controls was developed. Sensitivity and specificity of 92% and 69%, respectively, were achieved within this feasibility study. Those results proved EN's potentialities in discriminating between SARS-CoV-2 patients with respiratory failure and the control group.

An exploratory analysis included asymptomatic subjects who tested positive for SARS-CoV-2. Asymptomatic patients distinguished from the control group, suggesting that the proposed method can identify subjects positive to SARS-CoV-2 even if they do not present symptoms. Moreover, our preliminary results show that they present an intermediate response between patients with COVID-19 and respiratory failure and the control group. Such finding is very interesting because it suggests that the proposed method is sensitive both to the presence of an infection and the severity of the disease. Further studies are warranted to investigate whether the proposed method can be used to identify the treatable traits of respiratory failure or to monitor the progression of disease severity.

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