Titolo dello Studio:	Effect of nebulised saline on exhaled bio-aerosol particles and SARS-CoV-2 spreading in COVID-19 patients
Codice dello Studio:	
Numero Eudract:	
Versione del Protocollo:	Version No. 1.0,
Data:	May 15 2021
Finanziamenti:	
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Lista Centri Partecipanti [*se applicabile*]

Nome Centro	*Indicare nome, contatti degli sperimentatori afferenti al centro*
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Informazioni di Contatto

Nome Contatto Promotore	*Prof. Federico Lavorini* Dipartimento di Medicina Clinica e Sperimentale Università di Firenze
Nome Contatto per la Farmacovigilanza	*Indicare nome e contatti*

APPROVAZIONE DEL PROTOCOLLO

Gli Sperimentatori:

- approvano il presente Protocollo;
- dichiarano che lo studio verrà condotto in conformità a quanto riportato nel presente protocollo.

Prof. Federico Lavorini 03/06/2021

Prof. Gianmaria Rossolini 03/06/2021

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Prof. Francesca Buttini 03/06/2021

1. Background and study rationale

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COronaVirus Disease 2019 (COVID-19), has resulted in a worldwide pandemic and currently represents a major public health (1). The SARS-CoV-2 virus, an acute respiratory infectious agent, is primarily transmitted between people through respiratory droplets and contact routes (2,3). A recognized key to transmission of COVID-19, and droplet infections generally, is the dispersion of bio-aerosols from the patient. Bio-aerosols are defined as aerosols or particulate and matter of animal, plant or microbial origin with a range from 0.1 to >100 μm in diameter (4), that can contain viruses, bacteria, fungi, and are generated by infected persons when they cough, sneeze, talk, sing, or breathe. They can land in the mouth, nose, or eyes of those in proximity, and they have the potential to be inhaled into the lungs.

National and international guidelines recommend droplet/airborne and contact precautions for those caring for COVID-19 patients in ambulatory and acute care settings (5-9). An increased risk of transmission has been associated with aerosol generating procedures, including nebulised treatment. Despite the lack of evidence, there is currently a heightened concern regarding the potential risk of transmission of SARS-CoV-2 in the form of aerosolised respiratory droplets from patients with COVID-19 undergoing nebulised treatment (9,10). Studies regarding risk of spreading the virus in the environment during nebulisation are conflicting. For example, one study reports not to find the virus in hospital air samples near COVID-19 patients (11); in contrast, another study conducted using a manikin in a laboratory detected the virus at a distance of two meters (12). Despite the absence of any robust scientific evidence, some guidelines have advised against the

use of nebuliser treatment unless absolutely necessary, others have recommended the continued use of nebulised treatment when applicable (see 9 for further refs). Aerosols generated by a nebuliser is derived from a medication solution and is not a bioaerosol - potentially infectious - as that generated during cough or sneeze. However, nebulisers could generate fugitive emissions during the drug administration, which are added to patient expiration potentially infectious (9). Moreover, aerosol delivery devices, including nebulisers, could be theoretically considered as fomites, and, therefore, required strict hygiene rules before, during and after use (9). Interestingly, it has been reported that, in healthy volunteers, nebulisation of isotonic saline reduced the number of exhaled bio-aerosol particles expired by 72% for up to 6 hours due to modifications of the surface tension and viscous forces acting on lung-lining fluid (13). Whether nebulisation of isotonic saline aerosols may reduce exhaled particle also in patients affected by viral infection, including SARS-CoV-2, is unknown.

Hence, this project aims at investigating the effects of isotonic saline nebulisation in patients with SARS-CoV-2 infection. We hypothesise that isotonic saline nebulisation may transiently reduce the number of exhaled bio-aerosol droplets with no significant diffusion of virus in the environmental caused by the nebulisation.

2.0 Aims of the Study

The aims of the present projects are twofold:

1) To investigate the mass and size distribution of bio-aerosol particles before and after nebulisation of 0.9% saline in inpatients with COVID-19;

2) To assess the risk of SARS-CoV-2 spreading in the hospital patient's room environment after 0.9% saline nebulisation.

3. Methods

3.1 Study design

Single-center, cross-sectional, explorative study.

3.2 Study Duration

Estimated recruitment period: 4 months

Total study duration: approximately 6 months

3.3 Inclusion criteria

a) Patients of both genders, aged >18 years admitted to the Careggi University Hospital due to COVID-19;

b) Subjects capable of giving informed consent to participate, and available to comply with the requirements and procedures foreseen by the study protocol.

3.4 Exclusion criteria

a) Patients with COVID-19 requiring non-invasive or invasive mechanical ventilation;

b) Patients with COVID-19 requiring high (FiO_2 > 40%) level of supplemental oxygen or those requiring oxygen delivery via face mask;

c) Inability to provide a signed informed consent;

d) Any disease that, at the judgment of the investigator, may interfere with the study procedures.

3.5 Withdrawal of Subjects

Patients must be withdrawn under the following circumstances:

a) the patient wishes to be withdrawn (withdrawal of consent)

b) major protocol violation considered to be relevant by the Investigator

Whenever a patient is prematurely withdrawn from trial participation, the reason will be reported on the clinical report form.

3.6 Outcome endpoints

Endpoints will be the count of bio-aerosol exhaled particles within a preselected size-selective range (see protocol and study procedures), as well as changes in SARS-CoV-2 degrees in the hospital patient's room environment after 0.9% saline nebulisation.

3.7 Protocol and study procedures

All study procedures will be performed during 2 days at least 24 hours apart. Air sampling of the hospital patient's room environmental will be obtained according to the ISPE guidelines for the monitoring of pharmaceutical powders in working environments (14). In particular, five suction

XR5000 pumps (SKC Inc., Eighty Four, PA) containing a filter and running at 5 L/min for 15-20 minutes will be positioned around the patient's bed at different heights and distances (Figure 1) to allow control (i.e. before saline nebulisation) air samples collection. During the air-sampling period, patients will be free to talks and/or cough. After sampling for 15-20 min, the airborne viral load captured within the pump filter will be detected and quantified using a real-time polymerase chain reaction assay. The control air sampling will be repeated for three times at least 2 hours apart to obtain an average viral load. After sampling of control air, each patient will be requested to breathe normally for 3-5 min through a mouthpiece connected to a sampling T adapter, with one end of the T sampling connected to a six-channel optical particle counter (OPC, Climet, Ultimate 100, Redlansa, CA) to measure expired particle count and size. Each channel on the OPC tabulates particle counts within a size-selective range of six bins (0.3-0.5µm, 0.5-1 µm, 1-3 µm, 3-5 µm, 5-10 µm and >10 µm). The other end of the T sampling will be connected to a Delbag-Luftfilter air filter (COPULAR CKL Macropur-F Acelan, GEA, Berlin), for removal of any airborne particulates from the inhaled ambient air stream (Figure 2). All the above reported procedures will be repeated on the subsequent day before and 5, 30, 60 and 120 min after nebulisation of 0.9% saline delivered by means of a PARI LC Plus Jet nebuliser (PARI, Starnberg, Germany) connected to a compressed air source at 18 psi.

Cleaning and disinfection of the OPC will be carried out at the end of each investigation with vaporized hydrogen peroxide as suggested by Climet Instruments Company.

3.8 Sample size and data analysis

Due to the explorative nature of the study, a formal assessment of study power will not be performed. However, based on previous studies performed on healthy subjects (13), we estimate that about 10 patients will be sufficient to obtain the data. Parametric variables will be presented as mean ± SD, with their minimum, maximum and median, while the discrete data will be presented in contingency tables with percentages. Paired t-test will be used to assess differences in the particle size distribution as well as in the SARS-Cov-2 levels before and after nebulisation. The value for the statistical significance will be $P<0.05$.

4. Safety

All adverse events occurring during thorough the study will be recorded and documented by the Principal Investigator. Any serious adverse event will be reported as soon as possible to the independent Ethics Committee approving the study, according to the current local and national regulations. Expedite reporting will be performed by the Principal Investigator in case of any suspected, unexpected, serious adverse reaction or in case of other safety issues that qualify for expedite reporting, in accordance with the European Directive 2001/20/EC

5. Ethical Considerations

The protocol has been written, and the study will be conducted according to the Declaration of Helsinki and the ICH Harmonized Tripartite Guidelines for Good Clinical Practice. Furthermore, the study will be conducted in accordance with the Ministerial Decree of 17 December 2004.

The final protocol and the final version of the informed consent form will be approved by the local independent ethics committee (IEC) before the study can be commenced. The Principal Investigator is also responsible for informing the IEC of any amendment to the protocol. The Principal Investigator will provide the IEC with reports of Serious Adverse Events from the study site as per local and national requirements. The Principal Investigator will ensure that the patients are given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patients will be given the opportunity to ask questions and allowed time to consider the information provided. All patients will be requested to provide a signed and dated informed consent before conducting any study-related procedures.

6. Study Report and Publications

The study results may be published and/or presented at scientific meetings. A copy of the study report and of any further publication will be also sent to the IEC.

7. References

1. WHO. Coronavirus disease (COVID-19) technical guidance: Infection prevention and control. Geneva: World Health Organization. 2020. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-gui dance/infection-prevention-and-control. Accessed March 16, 2020

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6. American Association for Respiratory Care, Nebulizer Cleaning—Final State Survey Worksheets Released. Updated: April 20, 2017. https://www.aarc.org/nebulizer-cleaning.

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9. Lavorini F, Usmani OS, Dhand R. Aerosol delivery systems for treating obstructive airway diseases during the SARS-CoV-2 pandemic. Internal and Emergency Med 2021 In press

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12. Tang JW, Kalliomaki P, Varila TM, et al (2020). Nebulisers as a potential source of airborne virus. J Infect. 15

13. Edwards, D. A. *et al.* Inhaling to mitigate exhaled bioaerosols. P Natl Acad Sci Usa 101, 17383–17388 (2004).

14. Good Practice Guide: assessing Particulate Containment 2nd edition. At: Ispe.org/publications/guidance-documents/assessing-particulate-containment-performance.

15. Figure 1. Location of the five suction pumps for air sampling.

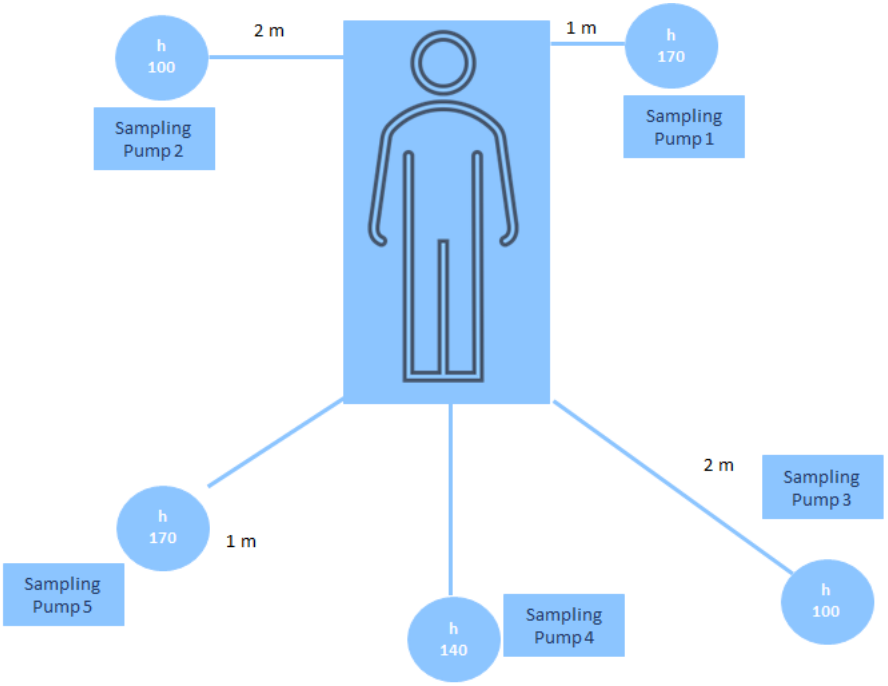

Figure 2. Set-up for assessment of mass and size of bio-aerosol exhaled particles