Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Airborne SARS-CoV-2 RNA excretion by patients with COVID-19 on different oxygen-delivery systems: a prospective observational study

M.L. Janssen, Y.P. Klazen, P. de Man, W. Hanselaar, D.S.Y. Ong, E.-J. Wils

Department of Intensive Care, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands
Department of Pulmonary Medicine, Erasmus MC, Rotterdam, the Netherlands
Department of Intensive Care, Erasmus MC, Rotterdam, the Netherlands
Department of Medical Microbiology and Infection Control, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands
Department of Pulmonary Medicine, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands
Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands

ARTICLE INFO

Article history:
Received 18 January 2022
Accepted 6 March 2022
Available online 12 March 2022

Keywords:
SARS-CoV-2
COVID-19
Air sampling
Viral load
High-flow nasal cannula

SUMMARY

Background: Concerns persist regarding the risk of airborne SARS-CoV-2 transmission by patients with COVID-19 on various modalities of oxygen therapy, such as high-flow nasal cannula (HFNC).

Aim: We aimed to compare the presence of airborne RNA in air samples between groups of patients with COVID-19 on different oxygen-delivery systems. We also explored factors that were associated with SARS-CoV-2 RNA positivity in air samples.

Results: Air samples were positive for SARS-CoV-2 RNA in three of 39 patients (8%) on HFNC, 0 of 13 (0%) on masks, versus five of 20 (25%) on nasal cannula. Odds ratio for air sample positivity was 0.52 (95% confidence interval (CI) 0.11 to 2.34) when comparing HFNC vs non-HFNC group, and 5.78 (1.24 to 27.01) for nasal cannula vs non-nasal cannula group. Patients with positive air samples in comparison with those with negative air samples were sampled earlier after symptoms onset (median: 7 vs 10 days; P=0.04) and had lower Ct values of diagnostic nasopharyngeal samples (median: 22 vs 26; P=0.02).

Conclusions: Air sample positivity was not related to oxygen support device but to viral load. These data suggest that the use of personal protection equipment should be based on risk management according to viral load rather than oxygen support device.

Introduction

Hypoxaemia in patients with coronavirus disease 2019 (COVID-19) can be treated with a variety of oxygen-delivery systems. Concerns, however, persist regarding their potential aggravating role in airborne severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) transmission. High-flow nasal...
cannula (HFNC) oxygen therapy appears clinically beneficial for patients with COVID-19 but was discouraged earlier in the pandemic because of its aerosol-generating potential [1,2]. Accumulating non-clinical data indicate that HFNC is not associated with more dispersion of aerosols and large droplets compared with conventional oxygen-delivery systems [3]. Studies on airborne SARS-CoV-2 RNA dispersion in patients with COVID-19 are, however, limited and have scarcely addressed the role of different oxygen-delivery systems. In the present clinical study, our main objective was to examine whether HFNC is associated with more frequency detection of SARS-CoV-2 RNA as compared with conventional oxygen-delivery systems [3].

**Methods**

This clinical study was performed from February to May 2021. The local institutional review board (IRB) declared that this study does not fall within the scope of the Dutch Medical Research involving human subjects act (IRB protocol number 2021–029). Patients were informed about the study and were asked for oral consent.

Inclusion criteria were: (1) adult SARS-CoV-2 polymerase chain reaction (PCR)-positive hospitalized patients for symptomatic hypoxaemia; (2) air sampling performed within 48 hours of the following diagnostic PCR; (3) receiving therapy with one of the following oxygen-delivery systems (Supplementary Figure S1): nasal cannula, non-rebreathing mask (NRM), air-entrainment mask (Intersurgical Ecolite™ with venturi valve), HFNC 40 L/min or 60 L/min (Airvo-2 System or Optiflow™ Nasal Cannula, Fisher & Paykel Healthcare). During the study period hypoxaemic patients with COVID-19 were initially treated by nasal cannula. If support was insufficient, treatment was escalated to HFNC, sometimes preceded by air-entrainment or NRMs. Dexamethasone was initiated in patients when therapy was escalated to oxygen administration, and a single dose of interleukin-6 receptor antagonist (tocilizumab) was administered when HFNC was started (as of February 2021).

The methodology of air sampling, RNA harvesting from filters and quantitative reverse transcription PCR (RT-qPCR) has been described previously [4]. In short, an IIR type surgical face mask (Romed Holland, type MASK-L) was used as sample filter placed on the hose inlet of a vacuum cleaner (Nilfisk household vacuum cleaner, with HEPA filter). Air samples were collected by investigators wearing personal protection equipment (PPE) in the ward or intensive care unit (ICU). All rooms were equipped with mechanical room ventilation with an air-exchange rate of six air changes per hour. Air was sampled for 2.5 min at two separate locations sequentially (Supplementary Figure S2): 50 cm behind and 30 cm above the patient’s head (dorsal sample; harvesting aerosols only) and 50 cm in front and 30 cm below (ventral sample; harvesting both droplets and aerosols). The marked circle of the sampling face mask was cut out, RNA was extracted using the Roche Magna Pure large volume total nucleic acid extracting kit.

Continuous data are presented as median with interquartile range. Categorical variables are reported as number with percentages. COPD, chronic obstructive pulmonary disease; Ct, cycle threshold; ICU, intensive care unit; PCR, polymerase chain reaction; VOC, variant of concern.

- Groups were compared using Mann–Whitney U-tests for continuous variables and Fisher’s exact test for categorical variables.

### Table I

Characteristics of patients with SARS-CoV-2 positive and negative air samples

|                             | Overall (N=75) | Positive air sample (N=8) | Negative air samples (N=67) | P<sup>*</sup> |
|-----------------------------|---------------|--------------------------|-----------------------------|--------------|
| Age in years                | 63 (51–72)    | 67 (63–75)               | 61 (49–72)                  | 0.12         |
| Male gender                 | 55 (73%)      | 5 (63%)                  | 50 (75%)                    | 0.43         |
| Hypertension                | 33 (44%)      | 5 (63%)                  | 28 (42%)                    | 0.29         |
| Diabetes Mellitus           | 22 (29%)      | 5 (63%)                  | 17 (25%)                    | 0.04         |
| Asthma                      | 4 (5%)        | 0 (0%)                   | 4 (6%)                      | 1.00         |
| COPD                        | 6 (8%)        | 1 (13%)                  | 5 (7%)                      | 0.50         |
| 4C Mortality score on admission | 10 (6–13)     | 11 (10–12)               | 9 (6–13)                    | 0.47         |
| Symptom duration until sampling in days | 10 (6–12)    | 7 (5–9)                  | 10 (7–13)                   | 0.04         |
| Ct value of diagnostic PCR  | 25 (22–30)    | 22 (20–24)               | 26 (22–30)                  | 0.02         |
| Sampling in ICU             | 23 (31%)      | 2 (25%)                  | 21 (31%)                    | 0.71         |
| Vaccination                 |               |                          |                             |              |
| None                        | 69 (92%)      | 62 (93%)                 | 7 (88%)                     | 1.00         |
| Single dose                 | 4 (5%)        | 4 (6%)                   | 0                           |              |
| Unknown                     | 2 (3%)        | 1 (2%)                   | 1 (13%)                     |              |
| COVID-19 variant            |               |                          |                             |              |
| Alpha                       | 55 (73%)      | 7 (87%)                  | 48 (72%)                    | 0.42         |
| Beta                        | 6 (8%)        | 0                       | 6 (9%)                      |              |
| Gamma                       | 3 (4%)        | 1 (13%)                  | 2 (3%)                      |              |
| No VOC                      | 5 (7%)        | 0                       | 5 (7%)                      |              |
| Unknown                     | 6 (8%)        | 0                       | 6 (9%)                      |              |

### Notes

- Continuous data are presented as median with interquartile range. Categorical variables are reported as number with percentages. COPD, chronic obstructive pulmonary disease; Ct, cycle threshold; ICU, intensive care unit; PCR, polymerase chain reaction; VOC, variant of concern.

- Groups were compared using Mann–Whitney U-tests for continuous variables and Fisher’s exact test for categorical variables.
Results

In total, 150 samples of 75 patients were analysed (Table I). Twenty patients were on nasal cannula, 13 on air-entrainment mask and NRM combined; three on NRM and 39 on HFNC (N = 19 flow 40 L/min; N = 20 patients flow 60 L/min). Patients were sampled in the ICU (31%) or respiratory ward (69%). As part of standard care, all patients received dexamethasone and 27 (69%) patients on HFNC received a single dose of tocilizumab prior to sampling. Four of 75 patients had received a first SAR-CoV-2 vaccination dose, whereas others were not (yet) vaccinated.

In total, eight patients (11%) had at least one positive air sample, either obtained at the ventral or dorsal sampling position. Positive dorsal and ventral air samples were equally distributed (five positive dorsal and five positive ventral samples: median Ct value 36 (interquartile range (IQR) 34–38). Two patients on nasal cannula had both a positive ventral and dorsal air sample. The median Ct-value of diagnostic PCR was lower in patients with positive air samples compared with patients with negative samples (median 22 (IQR 20–24) vs 26 (IQR 22–30); \( P = 0.02 \)). Patients with positive air samples were sampled earlier after onset of symptoms (median 7 (IQR 5–9) vs 10 days (IQR 7–13); \( P = 0.04 \)), and more frequently had diabetes mellitus (63% vs 25%; \( P = 0.04 \)). Of note, Ct-value of diagnostic PCR significantly correlated with the duration since symptom onset (Supplementary Figure S3). Environmental circumstances, patient’s behaviour and respiratory rate were similar between patients with positive and negative air samples (Table II). Median Ct-values of diagnostic PCR and symptom duration until sampling did not differ between groups on different oxygen-delivery systems. Air samples were positive in five of 20 patients (25%) on nasal cannula, in 0 of 13 patients (0%) on air-entrainment or NRM, and in three of 39 patients (8%) on HFNC (Table I). The proportion of patients with positive air samples was not higher for the HFNC group compared with different non-HFNC modality groups (Table III). In contrast, the proportion of positive samples was higher in the nasal cannula group compared with different non-nasal cannula groups.

Table II

Circumstances and patient’s behaviour in patients with SAR-CoV-2 positive and negative air samples

|                      | Overall (N=75) | Positive air sample (N=8) | Negative air samples (N=67) | \( P \) |
|----------------------|----------------|--------------------------|-----------------------------|-------|
| Days between diagnostic PCR and air sampling | 1 (1–1) | 2 (0–2) | 1 (1–1) | 0.23 |
| 24 h prior to air sampling: | | | | |
| NRS cough frequency | 4 (2–6) | 6 (3–8) | 3 (2–6) | 0.06 |
| Fisman cough severity score | 1 (1–2) | 1 (0–2) | 0 (0–1) | 0.16 |
| NRS sneeze frequency | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0.76 |
| Highest respiratory rate | 29 (24–35) | 34 (28–38) | 28 (24–35) | 0.21 |
| Lowest respiratory rate | 20 (16–20) | 20 (19–22) | 20 (16–20) | 0.25 |
| During air sampling: | | | | |
| Mouth open | 45 (60%) | 7 (87%) | 38 (57%) | 0.14 |
| Speaking | 44 (59%) | 7 (87%) | 37 (55%) | 0.13 |
| Sneezing | 2 (3%) | 1 (13%) | 1 (2%) | 0.21 |
| Coughing | 30 (40%) | 4 (50%) | 26 (39%) | 0.71 |
| Number of coughs | 0 (0–1) | 1 (0–3) | 0 (0–1) | 0.28 |
| Fisman cough severity score | 0 (0–2) | 1 (0–2) | 0 (0–1) | 0.37 |
| Respiratory rate | 24 (20–29) | 27 (21–30) | 24 (20–28) | 0.36 |
| Air sampling location | | | | |
| Intensive Care | 23 (31%) | 2 (25%) | 21 (31%) | 1.00 |
| Regular ward | 52 (69%) | 6 (75%) | 46 (69%) | 1.00 |
| Number of patients in the room | | | | |
| 1 | 41 (55%) | 2 (25%) | 39 (58%) | 0.23 |
| 2 | 22 (29%) | 3 (38%) | 19 (28%) | 0.80 |
| 3 | 6 (8%) | 1 (13%) | 5 (8%) | 0.76 |
| 4 | 2 (3%) | 0 | 2 (3%) | 0.56 |
| Unknown | 4 (5%) | 2 (25%) | 2 (3%) | 0.56 |

* Groups were compared using Mann–Whitney U-tests for continuous variables and Fisher’s exact test for categorical variables.

Discussion

This is the first real-life clinical study comparing SAR-CoV-2 RNA dispersion between different oxygen-delivery systems in a
large sample of hospitalized patients with COVID-19. In our analysis, the use of HFNC was not associated with more frequent detection of airborne viral RNA surrounding patients in a well-ventilated hospital environment. In contrast, the use of nasal cannula appeared to be associated with more frequent detection. An explanation for the observed difference may be the shorter duration between symptom onset and sampling, and higher nasopharyngeal viral load in patients on nasal cannula. Our airborne viral RNA data extend the evidence from imaging studies, arguing that HFNC does not enhance aerosols and droplet dispersion [3,6]. Ideally the next step would be to use viral culturing of air sample to compare the effect of different delivery systems more definitively. This technique, however, remains technically challenging and is currently not feasible for large-scale use, making viral RNA air sampling the most useful method currently available [7]. The observed correlation between high nasopharyngeal viral load (associated with a shorter duration of symptoms and nasal cannula use) and airborne viral RNA detection is in line with studies underscoring the role of high viral load early in the disease course and transmissibility [8]. Of note, in our previous study SARS-CoV-2 RNA was more frequently detected in up to 70% of samples obtained in poorly ventilated households of recently infected healthcare workers as compared with only 23% in a well-ventilated ICU-setting during potential aerosol-generating medical procedures [4]. We also observed that air samples were as frequently positive when obtained in the dorsal sampling position (where the contribution of large droplets is presumed negligible) as in ventral position supporting accumulating data on the role of aerosols as vectors of SARS-CoV-2 [9]. The patients included in our current study were predominantly infected with the alpha variant of concern (VOC), and only a minority had received a first dose of SARS-CoV-2 vaccine. The delta and omicron VOCs are associated with increased transmission rates, that possibly relate to the level of viral load (as measured by PCR or culture) in the upper airways. Ample evidence indicates that vaccination reduces transmission rate (as measured by PCR or culture) in the upper airways. Ample evidence indicates that vaccination reduces transmission rate but its relation to nasopharyngeal viral load is less clear [10–12]. The influence of vaccination and different VOC on viral aerosolization are important knowledge gaps that need to be addressed in future studies. These studies can take advantage of the easy-to-use air-sampling methodology as applied in the current study.

Several limitations of our study need consideration. First, this was a non-experimental clinical study precluding a direct comparison between oxygen-delivery systems with correction for confounders such as nasopharyngeal viral load and duration of symptoms. Although our sample size was considerable and the largest among similar studies, the event rate was too small for multivariable analysis. Nevertheless, we believe our real-life study is relevant as the strategy for escalating oxygen therapy mirrors contemporary clinical practice in COVID-19. Second, we could not investigate the association between the presence or quantity of airborne viral RNA and risk of transmission. Such investigation requires a larger sample size and meticulous contact-tracing. Third, we did not adjust our analysis for hazardous manoeuvres such as coughing, sneezing and vocation. Such manoeuvres, in addition to the level of ventilation and the level of patient’s infectivity may well be more relevant for viral transmission risk than the oxygen-delivery system itself [3,13,14]. In the current study, patients’ behaviour and environmental circumstances were similar for patients with positive versus negative air samples (Table II), suggesting no or only limited interference with our study results.

In conclusion, the risk of airborne SARS-CoV-2 RNA detection was not higher in patients on HFNC in comparison with other oxygen-delivery systems. More recent infection and higher viral load, at the moment of diagnostic sampling in patients on nasal cannula most likely contribute to the observed higher rate of viral dispersion. Our results emphasize that (in-hospital) use of PPE should be regarded equally important when nasal cannulas are used early during the disease course as compared with settings with possible aerosol-generating oxygen-delivery systems such as HFNC.

Acknowledgements

We would like to thank the medical microbiology laboratory technicians of the Franciscus Gasthuis & Vlietland hospital, in particular Han Veltman, Gerda Doejaaren, and Dick Wille, and team managers for their assistance in performing the experiments. We would like to thank Rene Bakker for the pictures used in Supplementary Figures S1 and S2. Informed consent was obtained from the persons in Supplementary Figures S1 and S2.

Author contributions

E.-J.W. takes responsibility for (is the guarantor of) the content of the manuscript, including the data and analysis. The following authors are responsible for the various
aspects of the manuscript, as indicated: P. de M., D.S.Y.O., E.-J.W.: conception of the work; M.L.J., P. de M., D.S.Y.O., E.-J.W.: design of the work; M.L.J., Y.K., P. de M., W.H., D.S.Y.O., E.-J.W.: acquisition, analysis, and interpretation of data for the work; M.L.J., Y.K., P. de M., W.H., D.S.Y.O., E.-J.W.: critical analysis and revision of the draft; M.L.J., Y.K., P. de M., W.H., D.S.Y.O., E.-J.W.: approved of the manuscript and are accountable for all aspects of the work.

Conflict of interest statement
The authors have no relevant financial or non-financial interests to disclose.

Funding sources
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2022.03.001.

References
[1] Ferioli M, Cisternino C, Leo V, Pisani L, Palange P, Nava S. Protecting healthcare workers from SARS-CoV-2 infection: practical indications. Eur Respir Rev 2020;29(155).
[2] Ospina-Tascon GA, Calderon-Tapia LE, Garcia AF, Zarama V, Gomez-Alvarez F, Alvarez-Saa T, et al. Effect of high-flow oxygen therapy vs conventional oxygen therapy on invasive mechanical ventilation and clinical recovery in patients with severe COVID-19: a randomized clinical trial. JAMA 2021;326(21):2161–71.
[3] Gaeckle NTLJP Y, Kreykes G, Evans MD, Hogan Jr CJ. Aerosol generation from the respiratory tract with various modes of oxygen delivery. Am J Respir Crit Care Med 2020;202(8):1115–24.
[4] de Man P, Ortiz M, Bluyssen PM, de Man SJ, Rentmeester MJ, van der Vliet M, et al. Airborne SARS-CoV-2 in home- and hospital environment investigated with a high-powered air sampler. J Hosp Infect 2021;119:126–31.
[5] Knight SR, Ho A, Pius R, Buchan I, Carson G, Drake TM, et al. Risk stratification of patients admitted to hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: development and validation of the 4C Mortality Score. BMJ 2020;370:m3339.
[6] Hamilton F, Arnold D, Bzdek BR, Dodd J, AERATOR group, Reid J, et al. Aerosol generating procedures: are they of relevance for transmission of SARS-CoV-2? Lancet Respir Med 2021;9(7):687–9.
[7] Pan M, Lednicky JA, Wu CY. Collection, particle sizing and detection of airborne viruses. J Appl Microbiol 2019;127(6):1596–611.
[8] Marks M, Millat-Martinez P, Ouchi D, Roberts CH, Alemany A, Corbacho-Monne M, et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. Lancet Infect Dis 2021;21(5):629–36.
[9] Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. Lancet 2021;397(10285):1603–5.
[10] Luo CH, Morris CP, Sachithanandham J, Amadi A, Gaston DC, Li M, et al. Infection with the SARS-CoV-2 Delta variant is associated with higher recovery of infectious virus compared to the Alpha variant in both unvaccinated and vaccinated individuals. Clin Infect Dis 2021 Dec 18. ciaa986.
[11] Mostaghimi D, Valdez CN, Larson HT, Kalinich CC, Iwasaki A. Prevention of host-to-host transmission by SARS-CoV-2 vaccines. Lancet Infect Dis 2022;22(2):e52–8.
[12] Puhach O, Adea K, Hulo N, Sattonnet P, Genecand C, Iten A, et al. Infectious viral load in unvaccinated and vaccinated patients infected with SARS-CoV-2 WT, Delta and Omicron. medRxiv 2022. https://doi.org/10.21203/rs.3.rs-1293087/v1.
[13] Chen PZ, Bobrovitz N, Premji Z, Koopmans M, Fisman DN, Gu FX. Heterogeneity in transmissibility and shedding SARS-CoV-2 via droplets and aerosols. Elife 2021;10: e70458.
[14] Somsen GA, van Rijn C, Kooij S, Bem RA, Bonn D. Small droplet aerosols in poorly ventilated spaces and SARS-CoV-2 transmission. Lancet Respir Med 2020;8(7):658–9.