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Airborne SARS-CoV-2 RNA excretion by patients with COVID-19 on different oxygen-delivery systems: a prospective observational study

M.L. Janssen [a,b,c], Y.P. Klazen [a], P. de Man [d], W. Hanselaar [e], D.S.Y. Ong [d,f], E.-J. Wils [a,c,*]

[a] Department of Intensive Care, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands
[b] Department of Pulmonary Medicine, Erasmus MC, Rotterdam, the Netherlands
[c] Department of Intensive Care, Erasmus MC, Rotterdam, the Netherlands
[d] Department of Medical Microbiology and Infection Control, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands
[e] Department of Pulmonary Medicine, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands
[f] Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands

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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) 0.11–2.34) when comparing HFNC vs non-HFNC group, and 5.78 (1.24–27.01) 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

* Corresponding author. Address: Kleiweg 500, 3045 PM, Rotterdam, the Netherlands.
E-mail address: e.wils@franciscus.nl (E.-J. Wils).

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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**
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%)	

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.

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 frequent detection of SARS-CoV-2 RNA as compared with other oxygen-delivery systems in air samples in the proximity of hospitalized patients with COVID-19. In addition, we explored what factors were associated with airborne viral RNA positivity.

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 diagnostic PCR; (3) receiving therapy with one of the following oxygen-delivery systems (Supplementary Figure S1): nasal cannula 2–6 L/min (Intersurgical Respiratory Systems), non-rebreathing mask (NRM) 15 L/min (Intersurgical Respiratory Systems, EcoLite™), air-entrainment masks (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. Sample filters were analysed on our validated in-house RT-qPCR assay on the presence of SARS-CoV-2 RNA and Cycle threshold (Ct) values were determined. Demographic, clinical, laboratory (including the 4C Mortality prognostic score [5]) and PCR data were recorded. Environmental circumstances, patients' behaviour and clinical condition were scored prior to and during sampling. The 24 hours prior to sampling cough severity was scored using the Fisman cough severity score, and cough and sneezing frequency by a Numerated Rating Scale (NRS; 0: no coughing/sneezing; 10: continuous coughing/sneezing). During sampling mouth opening, speaking, sneezing, coughing

Table II
Circumstances and patient's behaviour in patients with SARS-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%)	
Number of patients in the room				
1	41 (55%)	2 (25%)	39 (58%)	0.23
2	22 (29%)	3 (38%)	19 (28%)	
3	6 (8%)	1 (13%)	5 (8%)	
4	2 (3%)	0	2 (3%)	
Unknown	4 (5%)	2 (25%)	2 (3%)	

Patient's behaviour, such as mouth opening, speaking, coughing, sneezing and vital parameters such as respiratory rate were scored prior to and during sampling (see Methods). Continuous data are presented as median with interquartile range (IQR). Categorical variables are reported as number with percentages. NRS, numerated rating scale.

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

(yes/no; number of coughs/2.5 min; Fisman cough severity) and vital parameters such as respiratory rate were recorded.

Groups were compared using Mann–Whitney *U*-test for continuous variables and Fisher's exact test for categorical variables. Patients were divided into groups based on the oxygen-delivery system used during sampling. The following groups were additionally composed for comparative analysis: (a) non-HFNC: nasal cannula, air-entrainment mask and NRM combined; (b) non-nasal cannula: air-entrainment mask, NRM and HFNC combined; (c) non-air-entrainment/NRM: nasal cannula and HFNC combined. *P*-values below 0.05 were considered statistically significant. Analyses were performed with IBM SPSS Statistics for Windows Version 26.0 (IBM, Armonk, NY, USA).

Results

In total, 150 samples of 75 patients were analysed (Table I). Twenty patients were on nasal cannula, 13 on air-entrainment mask, 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 SARS-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 ventral and five positive dorsal 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.

Discussion

This is the first real-life clinical study comparing SARS-CoV-2 RNA dispersion between different oxygen-delivery systems in a

Table III
Air sample positivity risk: comparison between oxygen-delivery systems

	Odds ratio†	95% CI	P*
HFNC (*N*=39) vs. non-HFNC (*N*=36)	0.52	0.11–2.34	0.39
HFNC (*N*=39) vs. nasal cannula (*N*=20)	0.25	0.05–1.18	0.11
HFNC (*N*=39) vs. air-entrainment/NRM (*N*=13)	–	–	0.55
Nasal cannula (*N*=20) vs. non-nasal cannula (*N*=55)	5.78	1.24–27.01	0.03
Nasal cannula (*N*=20) vs. air-entrainment/NRM (*N*=13)	–	–	0.05
Air-entrainment/NRM (*N*=13) vs. non-air-entrainment/NRM (*N*=62)	–	–	0.19
ICU (*N*=23) vs. non-ICU environment (*N*=52)	0.73	0.14–3.92	0.14
HFNC: ICU (*N*=21) vs. non-ICU (*N*=18)	1.79	0.15–21.54	0.64

CI, confidence interval; HFNC, high-flow nasal cannula; NRM, non-rebreathing mask; ICU, intensive care unit.
* Groups were compared using Mann–Whitney *U*-tests for continuous variables and Fisher's exact test for categorical variables.
† Effect size estimates (odds ratio with 95% CI) to compare the risk of a positive air sample between groups.

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 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.

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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.

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Appendix A. Supplementary data

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