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Short Communication

Sensitivity and specificity of heat and moisture exchange filters sampling for SARS-CoV-2 detection in mechanically ventilated COVID-19 patients

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Abstract We assessed the sensitivity and specificity of SARS-CoV-2 detection by polymerase chain reaction in heat and moisture exchange filters (HMEF) in mechanically ventilated COVID-19 patients. We showed that testing HMEF might obviate the need for a tracheal sample to affirm that a patient is not ready to end isolation.

Introduction

The decision to discontinue isolation in critically ill mechanically ventilated COVID-19 patients, cannot be based on the resolution of the patients' symptoms. Consequently, respiratory samples, usually tracheal aspirates obtained by tracheal suction or bronchoalveolar liquid obtained by bronchoscopy, are frequently taken in COVID-19 patients

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treated by mechanical ventilation (MV) to monitor the progression of infection. Real time RT-PCR is used to measure SARS-CoV2 viral load and to detect the time at which the patient can be removed from isolation. However, open tracheal suction or bronchoscopy necessary for tracheobronchial secretions sampling are aerosol-generating procedures that expose care providers to contamination.[1,2]

The use of heat and moisture exchange filters (HMEF) is recommended for mechanically ventilated patients, unless the small dead space added to the ventilator circuit is seen detrimental.[3]

In our intensive care unit, we test mechanically ventilated COVID-19 patients for SARS-CoV-2 by RT-PCR twice a week. When HMEFs are used, they are changed at least at 72-hr intervals according to the manufacturer's instructions. It was therefore easy to make the HMEF changes and the tracheal suctions coincide without changing our routine practice and we took this opportunity to obtain simultaneous HMEF-tracheal sample pairs to conduct this study. HMEF fluid analysis has previously been shown to provide valuable insight of the distal lung airspace fluid composition.[4] Additionally, the value of testing HMEF by RT-PCR has previously been shown for the diagnosis of bacterial lung infection in ventilated patients.[5]

We investigated whether sampling HMEFs for SARS-CoV-2 detection by RT-PCR could replace tracheal aspirates.

Methods

According to the French law[6] our study did not change routine practice nor imposed new treatments, procedures, or additional biological samplings. The study protocol was approved by the ethic committee of the French intensive care society (#CE SRLF20-91) before the beginning of the study. Oral consent for the utilization of the data collected was obtained from all included patients or their family.

Patients and biological samples

Consecutive patients were prospectively included at any time during their ICU stay provided they were on invasive MV and had a HMEF (Humid-Vent™ Filter Compact, Teleflex® Medical Europe Ltd, Westmeath, Ireland [dead space of 38 mL]) inserted within their ventilator circuit.

HMEFs were changed at around 72-hr intervals according to the manufacturer's instructions.

At each HMEF change, an endotracheal aspirate was collected concomitantly by tracheal suctioning, according to local standardized operating procedure. A 10 Fr suction catheter was used, and 2 mL of sterile saline were instilled into the trachea before suctioning in all cases. The used HMEF and the tracheal secretion sample were sent to the hospital laboratory for SARS-CoV-2 RT-PCR.

The sampling of both the tracheal secretions and HMEF ended when a HMEF was no longer used, when the patient was extubated or when the patient died.

Pre-analytic processing of the HMEF

At the hospital laboratory, the patient's side of the HMEF was rubbed with a swab according to a standardized procedure described in eFig. 1. The swab was then placed in viral transport medium until analysis.

Real time RT-PCR

We used the TaqPath™ COVID-19 CE-IVD RT-PCR Kit (AppliedBiosystems, Thermo Fisher) and the QuantStudio™ thermocycler (AppliedBiosystems, Thermo Fisher). Three SARS-CoV-2 genes are targeted: Open reading frame 1 ab (*ORF1ab*), Spike protein (*S*) and Nucleocapside protein (*N*). A cycle threshold (Ct) $\leq$37 is considered positive for the detection of viral targets.[7] The laboratory declares the test positive when a Ct $\leq$ 37 is found for at least one target.

For the purpose of the study, indeterminate Ct were assigned the value of 40.

Sample size

For this "proof-of-concept" study we opted for the inclusion of a convenience sample of at least 20 patients and 100 HMEF-tracheal pairs.

Analysis of data

Sensitivity, specificity, and positive predictive value (PPV) of a positive HMEF RT-PCR test to predict the positivity of the tracheal RT-PCR test were calculated as the mean of the variable of interest in 2000 non-stratified bootstrap replicates of the study population. Lower and upper bounds of the 95% confidence interval (95%CI) were defined as the 2.5% and 97.5% percentile of each variable. Sensitivity and specificity of a HMEF sample Ct $\leq$ 37 for detecting a tracheal sample Ct $\leq$ 37 for each gene were calculated on raw data (i.e., without bootstrapping) and given with their 95%CI.

The limits of agreement between the Ct values obtained with the HMEF and the corresponding tracheal samples, the mean bias (HMEF Ct minus tracheal Ct) and its standard deviation (SD) were calculated by linear mixed-effect modelling for each gene, assuming that the serial measurements made in each patient had an autoregressive correlation structure and that patients had random intercept.[8] The 95%CI of the mean bias and of the lower and upper limits of the agreement interval were defined as the 2.5% and 97.5% percentile of the variable of interest calculated on 2000 non-stratified bootstrap replicates of the study population. The minimal Ct among Ct for the *ORF1ab*, *N* and *S* genes obtained with the tracheal sample were also compared to the minimal Ct obtained with the corresponding HMEF. Results are shown on Bland—Altman plots.

The tracheal/HMEF ratio of gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method[9] and expressed on a decimal logarithm scale.

To search for factors potentially interfering with the tracheal/HMEF ratio of gene expression, we used linear mixed modelling: the tracheal/HMEF ratio of N-gene expression was the dependent variable; patients were entered as a variable with random intercept; the following variables were entered successively in the model as variables with fixed effect: the duration of use of each HMEF, the time order of each sample pair, and respiratory variables measured before each sampling (tidal volume [expressed in mL/predicted body weight (kg)], respiratory rate, minute ventilation, end-expiratory pressure and use of nebulized nitric oxide). The model with the combination of fixed effect variables that explained the largest amount of variance was selected by the likelihood ratio test.

A 2-tailed P-value <0.05 was considered statistically significant. All analyses were conducted with R v. 4.0.2 (R Foundation, Vienna, Austria).

Results

From 16th December 2020 to 27th January 2021, we included 27 patients (see eTable 1 for patients' characteristics) and obtained 130 pairs of HMEF and tracheal samples.

The settings of the ventilator and the use of nebulized therapy for each patient at each sampling are summarized in eTable 2.

The number of tracheal/HMEF pairs analyzed per patient (median [IQR]) was 4 [2; 8] (Range: 1–13).

The duration of use of the analyzed HMEF (median [IQR]) was 70.0 h [60.8; 77.5] (Range: 16.7–171.0].

Overall, 57/130 (43.8%) HMEF RT-PCR and 86/130 (66.2%) tracheal RT-PCR were declared positive.

Bootstrapped sensitivity, specificity, and PPV of a positive HMEF RT-PCR test to predict the positivity of the tracheal RT-PCR test were 62.8% (52.4; 73.2), 93.2% (85.0; 100), and 94.8% (88.3; 100) respectively.

A HMEF *N*-gene Ct $\leq$ 37 had a sensitivity of 64.3% (53.1; 74.2) and a specificity of 95.6% (84.0; 99.2) for detecting a tracheal Ct $\leq$ 37. We found similar results for the other genes (Table 1).

The limits of agreement between HMEF and tracheal Ct values were −5.8 (−7.5; −4.3) to 15.1 (12.9; 17.2) for the *N* gene (Fig. 1). Similar agreement intervals were found for the other genes (eFigures 2, 3 and 4).

The mean (SD) tracheal/HMEF ratio of *N*-gene expression was 1.9 (SD: 2.1) log.

The search for factors that best explained the tracheal/HMEF ratio of gene expression provided no useful information (see eTable 3 and eFig. 5).

Discussion

Ct values obtained from HMEF and tracheal aspirates are not interchangeable as tracheal aspirates contain much more viral material. A recent report in 4 COVID-19 ventilated patients who underwent a paired HMEF/tracheal sampling, showed that only 1 patient had a positive RT-PCR for SARS-CoV-2 on the HMEF.[10] This suggested that sampling of the HMEF had a very low sensitivity. However, we found a higher sensitivity of 62.8% and a specificity >93% (Table 1),

Table 1 Sensitivity, specificity, and positive predictive value of HMEF Ct $\leq$ 37 to detect a tracheal Ct $\leq$ 37.

N gene		
	Ct trachea ≤37	Ct trachea >37
Ct HMEF ≤37	54	2
Ct HMEF >37	30	44
Sensitivity: 64.3% (53.1–74.2)		
Specificity: 95.6% (84.0–99.2)		
Positive predictive value: 96.4% (86.6–99.4)		
S gene		
	Ct trachea ≤37	Ct trachea >37
Ct HMEF ≤37	43	1
Ct HMEF >37	41	45
Sensitivity: 51.2% (40.1–62.2)		
Specificity: 97.8% (87.0–99.9)		
Positive predictive value: 97.7% (86.5–99.9)		
ORF1a gene		
	Ct trachea ≤37	Ct trachea >37
Ct HMEF ≤37	46	3
Ct HMEF >37	38	43
Sensitivity: 54.8% (43.6–65.5)		
Specificity: 93.5% (81.1–98.3)		
Positive predictive value: 93.9% (82.1–98.4)		
Lowest Ct among N, S, and ORF1a genes		
	Ct trachea ≤37	Ct trachea >37
Ct HMEF ≤37	54	3
Ct HMEF >37	32	41
Sensitivity: 62.8% (51.6–72.8)		
Specificity: 93.2% (80.3–98.2)		
Positive predictive value: 94.7% (84.4–98.6)		

Ct, cycle threshold; *HMEF*, Heat and moisture exchange filter.

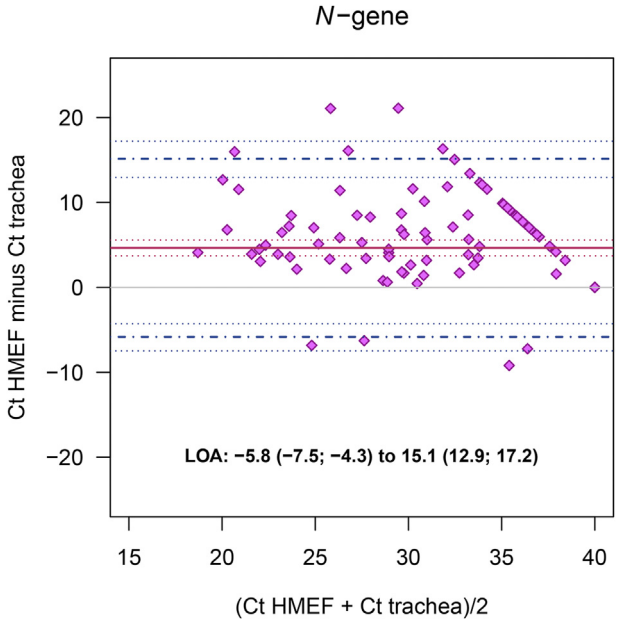

Figure 1. Title: Bland–Altman plot comparing the Cycle threshold value obtained on the HMEF and the tracheal samples. Legend: *Ct*, cycle threshold; *HMEF*, heat and moisture exchange filter; *LOA*, limits of agreement.

which may be due to different pre-processing of the HMEF material (which could be further explored).

This study has several limitations. First, it was a small-sized, single-center pilot study. Second, despite standardization of the procedure of tracheal suctioning, the volume of tracheal secretions collected could vary. Some samples may have been more diluted than others. This may have induced some false negative tracheal RT-PCR tests and slightly biased the sensitivities and specificities we calculated.

Based on the high specificity we observed, a HMEF RT-PCR positive result, which occurred in 43.8% of instances, might obviate the need for a tracheal aspirate to affirm that a patient is not ready to end isolation. If confirmed in larger studies involving different ICUs, this may avoid a significant number of long disconnections from the respirator necessary for open tracheal suction and minimize the risk of care givers contamination.

Ethics statement

As the study did not change routine practice nor imposed new treatments, procedures, or additional biological samplings, it was considered not to involve the human person, in the meaning of the French law [8,9], but only health data. Hence, submission of the protocol to the national competent authorities and registration in a trial registry were not required. The study protocol was approved by the ethic committee of the French intensive care society (#CE SRLF20-91) before the beginning of the study. Written and oral information was given to patients and their family. Oral consent for the utilization of the data collected was obtained from all included patients or their family.

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Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

All authors report no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2022.04.002.