

# Use of exhaled breath condensate (EBC) in the diagnosis of SARS-CoV-2 (COVID-19)

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## ABSTRACT

False negatives from nasopharyngeal swabs (NPS) using reverse transcriptase PCR (RT-PCR) in SARS-CoV-2 are high. Exhaled breath condensate (EBC) contains lower respiratory droplets that may improve detection. We performed EBC RT-PCR for SARS-CoV-2 genes (E, S, N, ORF1ab) on NPS-positive (n=16) and NPS-negative/clinically positive COVID-19 patients (n=15) using two commercial assays. EBC detected SARS-CoV-2 in 93.5% (29/31) using the four genes. Pre-SARS-CoV-2 era controls (n=14) were negative. EBC was positive in NPS negative/clinically positive patients in 66.6% (10/15) using the identical E and S (E/S) gene assay used for NPS, 73.3% (11/15) using the N/ORF1ab assay and 14/15 (93.3%) combined.

## INTRODUCTION

COVID-19 is routinely diagnosed by detection of SARS-CoV-2 viral RNA via reverse transcription PCR (RT-PCR) from nasopharyngeal swabs (NPS).<sup>1</sup> Target genes include spike surface glycoprotein (S), small envelope protein (E), nucleocapsid protein (N) and RNA dependant RNA polymerase (ORF1ab).<sup>2</sup> WHO guidelines recommend identification of one of these targets for diagnosis of COVID-19 in high prevalence areas.<sup>3</sup> Negative NPS results do not outrule infection and in patients with a suspicion of COVID-19 and negative NPS, lower respiratory tract sampling is recommended.<sup>4</sup> Bronchoalveolar lavage and tracheal aspirates are invasive and impractical in COVID-19. Novel, non-invasive options representing the lower respiratory tract should be explored.

Exhaled breath condensate (EBC) contains respiratory droplets representing the lower airways. We have previously detected DNA mutations in EBC from patients with lung cancer,<sup>5</sup> and EBC RT-PCR has been used to identify respiratory viruses including coronavirus.<sup>6</sup> We hypothesised that EBC can detect SARS-CoV-2 and aid diagnosis in NPS-negative patients with suspected COVID-19.

## METHODS

This was a prospective, observational, proof-of-concept study. Patients were recruited from the hospital COVID-19 pathway (designed to triage patients with a potential diagnosis of COVID-19) between 6 April and 8 May 2020. Patients were recruited if they could give informed written

consent, perform EBC testing and had clinical and radiological evidence of COVID-19 or a clear alternative diagnosis. Forty patients were recruited and divided into three groups: (1) SARS-CoV-2 NPS positive, (2) NPS negative with a clinical diagnosis of COVID-19 and (3) NPS negative with other clinical diagnoses. Clinical diagnosis of COVID-19 was confirmed using expert clinical opinion and characteristic imaging.<sup>7</sup> Fourteen samples obtained before the emergence of SARS-CoV-2 from patients with lung cancer were included as negative controls. Where possible, we serologically tested patients who were NP-swab negative with a clinical diagnosis of COVID-19 to validate our results.

## Sample collection and processing

EBC was collected using the RTube condenser (Respiratory Research Inc, Charlottesville, Virginia, USA). Patients were instructed to breathe through the mouthpiece for 2 min. RNA was extracted from 140 µL of EBC using the QIAamp viral RNA mini kit (Qiagen) and eluted in 50 µL of buffer. RT-PCR for the E and S genes (E/S) was performed using the RealStar SARS-CoV-2 RT-PCR kit (Altona Diagnostics), as used for the NPS. The N and ORF1ab (N/ORF1ab) genes were analysed using the COVID-19 (SARS-CoV-2) Triplex RT-qPCR detection kit (Assay Genie). Assays were performed on the Applied Biosystems 7500 instrument. EBC was deemed positive for SARS-CoV-2 if at least one of the four genes tested positive. Serological analysis was performed using the anti-SARS-CoV-2 antibody test (Roche Diagnostics).

## RESULTS

### Patients

Of the 40 patients recruited, 16 (40%) were NPS positive, 15 (37.5%) were NPS negative (median negative NPS 2, range 2–3) with a clinical diagnosis of COVID-19 and 9 (22.5%) were NPS negative with other clinical diagnoses including pulmonary emboli (2), new lung cancers (2), cavitating pneumonia (1), pulmonary nodules (1), and three patients with normal lung imaging. One of these had methicillin-sensitive *Staphylococcus aureus* septicaemia and two had costochondritis. Baseline characteristics of prospectively enrolled patients are included in table 1. Nine out of 15



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**Table 1** Clinical characteristics of NPS positive, NPS negative/clinically positive and NPS negative/other clinical diagnoses

| Clinical characteristics                        | SARS-CoV-2 NPS positive (n=16) | SARS-CoV-2 NPS negative/clinically positive (n=15) | SARS-CoV-2 NPS negative/other diagnosis (n=9) |
|---|--------------------------------|--|---|
| Median age (range)                              | 40.5 (31–69)                   | 68 (32–95)   | 47 (27–73)                                    |
| No. of male (%)                                 | 12 (75)                        | 13 (86.6)  | 3 (33)  |
| Median BMI (range)                              | 28 (20.2–37.9)                 | 26.35 (23–28.4)                                    | 26.8 (23–30)                                  |
| Smokers (%)                                     | 3 (18.7)                       | 6 (40)   | 4 (44)  |
| Lab results (range)                             |                                |  |   |
| Ferritin ng/mL (30–400)                         | 1182 (224–2705)                | 875 (316–21 063)                                   | 336.5 (256–1979)                              |
| Lactate dehydrogenase IU/L (135–214)            | 333 (276–794)                  | 262 (162–825)                                      | 310.5 (190–322)                               |
| D-dimer ug/mL (0–0.5)                           | 1.03 (0.9–35)                  | 1.26 (0.42–15)                                     | 3.33 (0.89–71.4)                              |
| PaO <sub>2</sub> kPa (11.1–14.1)                | 8.9 (7.5–11.5)                 | 8.9 (7.8–12)                                       | 11.1 (7–16.5)                                 |
| C reactive protein mg/L (0–5)                   | 166.5 (15–274)                 | 166 (37.3–293)                                     | 47 (1–308)                                    |
| Lymphocytes 10 <sup>9</sup> /L (1–4)            | 0.81 (0.4–1.67)                | 0.86 (0.26–1.11)                                   | 1.63 (0.62–1.52)                              |
| Troponin ng/L (0–13)                            | <14 (14–58)                    | <14 (14–54)  | <14 (14–75)                                   |
| Median temperature (range)                      | 37.85 (35.9–41)                | 37.1 (36.4–39)                                     | 37 (36.1–37.8)                                |
| Median number of NPSs (range)                   | 1 (1)                          | 2 (2–3)  | 1 (1–3)                                       |
| Median no. of days symptomatic pre-NPSs (range) | 10 (4–20)                      | 7 (3–16)   | 5 (2–10)                                      |
| NPS/EBC interval – median (range) (days)        | 3 (1–19)                       | 1 (0–7)  | 1 (0–5)                                       |

EBC, exhaled breath condensate; NPS, nasopharyngeal swab.

patients with negative NPS but clinical diagnosis of COVID-19 had serology performed. Of the remaining six, three had died, two did not consent to testing and one was uncontactable.

### Exhaled breath condensate

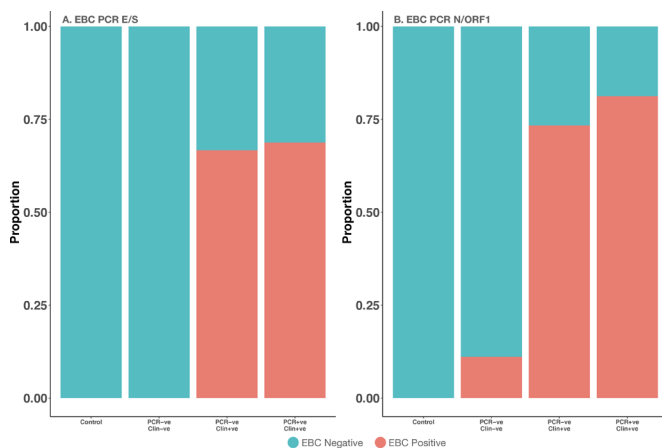
In patients with a positive NPS and those with a negative NPS but clinical diagnosis of COVID-19, EBC RT-PCR was positive for SARS-CoV-2 in 21/31 (68%) of cases using the identical two-gene (E/S) assay kit used for NPS testing. Detection increased to 29/31 (93.5%) when EBC RT-PCR was performed using four gene targets (S/E/N/ORF1ab). In the clinically positive but NPS-negative group, EBC was positive in 66.6% (10/15) using the E/S assay (figure 1A), 73.3% (11/15) using the N/ORF1ab assay (figure 1B) and 93.3% (14/15) using the

four genes. Eight of the nine (89%) NPS negative with other clinical diagnoses patients had negative EBC. One patient with a new diagnosis of lung cancer tested positive despite low clinical suspicion. None of the 14 samples in the pre-SARS-CoV-2 era control group tested positive. Serology testing was carried out on patients with a negative NPS but clinical diagnosis of COVID-19 at a median of 34 (range 24–51) days post-symptom onset. Six out of nine (66.6%) patients had positive antibodies. All nine corresponding EBC samples were positive for SARS-CoV-2.

### DISCUSSION

Our study supports the hypothesis that EBC can identify SARS-CoV-2 by RT-PCR and that testing multiple genes together increases detection. False negative rates approaching 30% are reported using NPS RT-PCR,<sup>8</sup> with detection dropping up to 40% after 5 days of symptoms.<sup>9</sup> For our study, the median number of days patients were symptomatic prior to admission was 7 (range 2–20). Despite this, EBC detected SARS-CoV-2 in 66.6% (10/15) (E/S assay), 73.3% (11/15) (N/ORF1ab assay) and 93.3% (14/15) (all four genes) of NPS-negative patients with a clinical diagnosis of COVID-19.

The study has limitations. Power calculations indicated a need for 155 samples to detect a statistically significant difference between NPS and EBC RT-PCR using the same E/S assay with an 80% power and alpha value of 0.05. However, increased sample size was not possible due to decreased admissions in response to public health measures. EBC RT-PCR failed to identify SARS-CoV-2 in 5/16 (31%) NPS-positive cases using the identical E/S assay, suggesting EBC should be used as an adjunct rather than a replacement for NPS RT-PCR. Owing to strict laboratory access restrictions and some patients being initially too unwell to provide samples, EBC collection occurred a median of 2 days (range 0–19) after NPS testing, potentially introducing bias favouring EBC detection. Three out of nine (33.3%) patients had no evidence of antibodies to COVID-19 despite having positive EBC RT-PCR. However,



**Figure 1** Exhaled breath condensate (EBC) reverse transcriptase PCR for SARS-CoV-2. The proportion of positive and negative results for EBC reverse transcriptase PCR (EBC RT-PCR) for SARS-CoV-2 in the different clinical cohorts are shown for (A) E and S genes and (B) N and ORF1ab genes. Figure 1A also highlights the proportion of NPS-positive patients that tested negative using EBC RT-PCR, 5/16 (31%).

median time to serology was 34 days (range 34–51), and currently few data are available to confirm continued antibody response 35 days post infection, particularly in milder cases.<sup>10</sup>

In conclusion, this study provides promising results that EBC RT-PCR is an effective, non-invasive method of identifying SARS-CoV-2 from lower respiratory tract samples and should be considered to aid diagnosis of COVID-19 in patients with a high suspicion of infection but negative NPS.

**Contributors** DR and ST: joint first authors responsible for drafting, conception, design, acquisition, analysis and interpretation. SM: data analysis and interpretation. MC, OB, PGM and LG: data acquisition and analysis. EDB, PB, RWC, KH, CG, NGM, MEO and IS: data acquisition and analysis. RM and BH: joint senior authors responsible for conception, design and drafting of the manuscript for important intellectual content.

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