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CHEST

Paired Nasopharyngeal and Deep Lung Testing for Severe Acute Respiratory Syndrome Coronavirus-2 Reveals a Viral Gradient in Critically Ill Patients

A Multicenter Study

To the Editor:

Since the start of the coronavirus 2019 (COVID-19) pandemic, arising from Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) viral infection, approximately 13,000 patients have been admitted to critical care in the United Kingdom; most have required advanced respiratory support.[1]

Samples for SARS-CoV-2 detection can be obtained from the upper (nasopharyngeal/oropharyngeal swabs) or lower respiratory tract (sputum/endotracheal aspirate/BAL).[2] Viral RNA is detected using reverse transcriptase polymerase chain reaction (RT-PCR). The Cycle threshold (Ct) has a simple negative linear correlation with the logarithm of the number of gene copies in the original sample and thus can be used to provide a semiquantitative estimate of the viral RNA in a specimen.[3]

SARS-CoV-2 has been suggested to be shed predominantly from the upper respiratory tract, distinguishing it from SARS-CoV-1, in which replication occurs mainly in the lower respiratory tract.[4-6] A recent multi-site viral detection study[5] indicated higher nasopharyngeal (NP) viral loads in some patients early in the course of disease, although they generally detected viral RNA in sputum for longer. However, this study[5] was conducted on patients with mild disease, and whether the results pertain to critically ill patients is unclear.

Our objective was to evaluate SARS-CoV-2 RNA loads between paired NP and deep lung (endotracheal aspirate or BAL) samples from critically ill patients.

Methods

Patients admitted to five ICUs in three UK hospitals with PCR-confirmed COVID-19 were identified retrospectively. The sites were Addenbrookes Hospital Cambridge, a tertiary center with three units (a general ICU, neurotrauma unit, and dedicated COVID unit), Royal Papworth Hospital Cambridge (tertiary respiratory failure and extracorporeal membrane oxygenation [ECMO] unit), and Sunderland Royal Hospital (general unit), a large district general hospital. Patients with paired NP and deep lung samples were identified, with "paired" defined as samples taken within 24 hours of each other. At Addenbrooke's and Royal Papworth, samples were analyzed in a common microbiology laboratory, using in-house RT-PCR after extraction using the Easy Mag platform (Biomeriuex, Basingstoke, UK). Samples from Royal Sunderland were analyzed using the Cobas 6800 (Roche, Welwyn, UK). Clinical data were obtained from case note review. Comparison of paired data was by Wilcoxon test, regression was by simple linear regression, analysis was conducted using Prism (v8.4.3 for MacOS, Graphpad). As retrospective service evaluations of anonymized routinely collected data, the requirement for research ethics committee review and consent was waived, and each site registered their service evaluation internally.

Results

Fifty-two patients with paired samples were identified. The median age of the patients was 49 years (range, 24-74), with 74% males. The median duration between onset of symptoms and when paired samples were obtained was 8 days (range, 1-24). When paired samples were obtained, one patient was receiving facemask oxygen, six patients were receiving noninvasive ventilation or mask CPAP, 27 patients were mechanically ventilated, and 18 were on ECMO.

There was a significant gradient between NP and deep lung viral loads (Fig 1A), with median Ct value of 29 for NP and 24 for deep lung samples. Of 52 paired samples, 17 were negative on NP swabs but positive on deep sample, whereas two were negative on deep sample but positive on NP (both patients were on ECMO). Of the subgroups by ventilatory support, the 27 mechanically ventilated patients demonstrated the largest gradient (Fig 1B-D). Although there was no apparent relationship between time between symptom onset and Ct value in

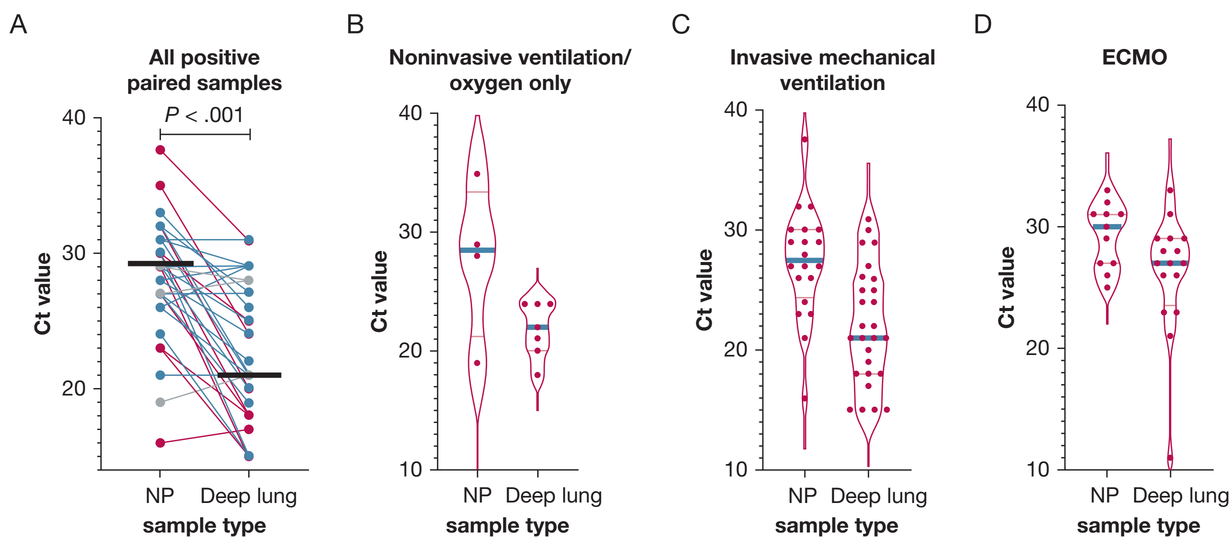

Figure 1 – *Comparison of viral load of samples from nasopharynx (NP) and deep lung (endotracheal aspirate or BAL). A, Paired samples excluding samples in which NP (16) or deep lung (2) were below the limit of detection; color indicates time from symptom onset to test (red, <7 days; blue, 7-13 days; gray, >13 days); black line indicates median value.* P *value by Wilcoxon matched-pairs test. B, All samples from patients receiving noninvasive ventilation or oxygen at time of test. Blue line indicates median value. C, All samples from patients receiving invasive mechanical ventilation at time of test. Blue line indicates median value. D, All samples from patients receiving ECMO at time of test. Blue line indicates median value.*

NP swabs, deep lung samples tended toward higher Ct values the later they were taken (Fig 2A, 2B). Consequently, the NP-lung gradient was smaller in patients sampled later in their disease course (Fig 2C). ECMO patients tended to be sampled later after symptom onset than invasive and noninvasively ventilated patients (median duration from symptom onset to test was 12 days for ECMO, 7 for invasive mechanical ventilation, and 8 for noninvasive ventilation/oxygen).

Discussion

In our case series of critically ill patients with COVID19, NP swabs were relatively insensitive for detection of SARS-CoV-2: 67% of NP samples detected viral RNA, compared with 96% of deep respiratory samples. There was also a clear viral gradient with a median five cycle (9 cycles for mechanically ventilated) lower Ct value in the lungs. To the best of our knowledge, this is the first report of paired respiratory samples from critically ill patients with COVID-19.

In SARS, arising from SARS-CoV-1, there was a substantial rate of false-negative nasopharyngeal swabs,[6] leading to the suggestion that SARS-CoV-1, unlike SARS-CoV-2, had a predilection for lower airways.[4] Our work challenges this assumption, demonstrating significantly higher viral loads in the lower respiratory tract amongst critically ill patients. Possibly the higher viral load in the lungs may contribute to the harmful

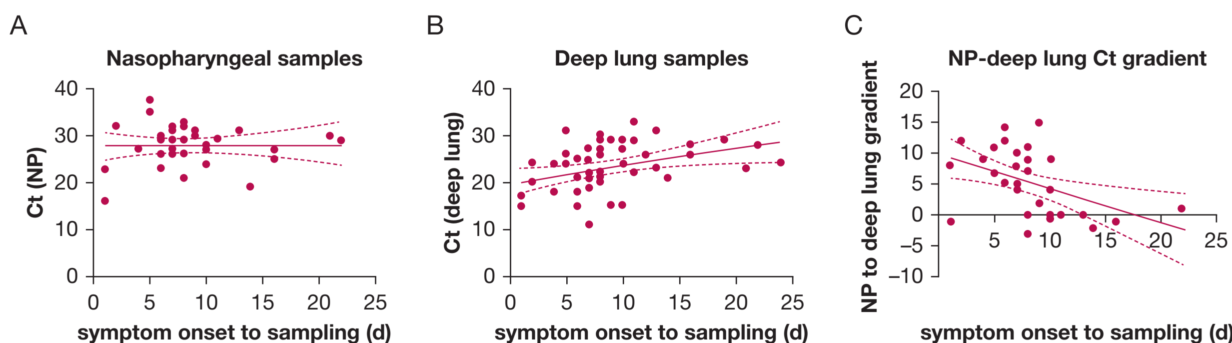

Figure 2 – *Relationship between duration of symptoms to testing and Ct value. A, Positive nasopharyngeal swab Ct values, $R^2 = 0$,* P *= .89. B, Positive deep lung sample Ct values, $R^2 = 0.14$,* P *= .0075. C, NP to deep lung Ct gradient (where both samples were positive), $R^2 = 0.2$,* P *= .0089. Lines show regression line from simple linear regression with 95% CIs. Ct = cycle threshold.*

inflammatory response that constitutes the pathology of SARS. Interestingly, Lucas and colleagues[7] report that patients with severe COVID-19 did not clear their nasopharyngeal carriage, which is consistent with our findings (Fig 2A).[7] They also found that initial NP viral load did not correlate with severity of illness[7]; however, they did not report lung viral loads. Our finding of lower lung viral loads at later timepoints, especially in patients with the most severe respiratory failure requiring ECMO, suggests that viral clearance is not sufficient to ameliorate the pulmonary inflammatory response. This may explain why later (>7 days after symptom onset) corticosteroids improve pulmonary function in severely affected COVID-19 patients.[8]

Although there is understandable concern about the risk of deep lung sampling such as BAL or endotracheal aspirate generating aerosol and increasing risk of health care worker infection, our experience during the pandemic is that such procedures can be performed safely. Use of enhanced aerosol protecting personal protective equipment, respiratory isolation, and closed/semi-closed respiratory circuits combine to increase the safety of this process.

This study reports from five units using two different molecular tests, increasing the generalizability of our findings. The retrospective nature of the data collection may introduce a source of bias against NP testing, which is often used as the first-line test, and that deep respiratory sampling may have only been undertaken if RNA was not detected from NP swabs. However, during the first wave of the pandemic, the turn-around time for PCR was generally greater than 24 hours, and it is unlikely that negative NP swabs will have influenced the decision to obtain paired samples within the same 24-hour period. Although all NP swabs were taken by nurses appropriately trained in viral sample acquisition, we cannot be certain that the patient's nasopharynx was correctly sampled in all cases; however, it does reflect the real-world experience of virological sampling. Possibly the gradient found reflects technical rather than biological factors, because both tracheal washing and BAL will sample a much larger surface area than an NP swab, although both of these former techniques will dilute respiratory lining fluid, which may have the opposite effect. The finding of a temporal relationship in the gradient noted supports our contention that this is a genuine biological finding. Irrespective of this, the clinical implication that deep lung samples are more sensitive amongst critically ill patients remains a key finding.

In conclusion, we have found that critically ill patients with COVID-19 demonstrate a significant viral gradient from the upper to the lower respiratory tract, which may have diagnostic and pathophysiological importance. We conclude that, in the absence of an existing positive result, lower respiratory tract samples should be obtained for the detection of SARS-CoV-2.

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