COVID-19: Extensive epithelial damage and ciliary dyskinesia in hospitalised patients*

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Rhinology 60: 0, 0 - 0, 2022

https://doi.org/10.4193/Rhin21.233

***Received for publication:**

June 22, 2021

Accepted: December 20, 2021

Dear Editor:

Infection with SARS-CoV-2 can cause severe respiratory disease and it is predicted that the COVID-19 pandemic will leave a substantial number of patients with long-term respiratory complications [(1)].

The ciliated respiratory epithelium is one of the main sites of SARS-CoV-2 infection. ACE2, the major receptor for SARS-CoV-2 is expressed mainly on ciliated cells [(2)], and initial cell culture reports suggested that the virus preferentially replicates in ciliated epithelial [(3, 4)] cells reducing mucociliary clearance [(4)]. However, Zhu and colleagues [(2)] have shown that both secretory and ciliated cells are infected by SARS-CoV-2 with peak viral production 48-72 hours post infection [(2)]. Electron microscopy of infected cultures 72 hours post infection suggested that secretory cells release vesicles containing large numbers of viral particles by exocytosis and demonstrated the presence of unique plaque like cytopathic areas that when observed in live culture lacked ciliary beating. Abnormal mitochondria and extensive cell death were associated with the plaques that expanded during infection and syncytial cell formation was also observed [(2)].

Post-mortem examination following COVID-19 has revealed epithelial damage in the trachea with exposure of basal cells to the airway lumen and extensive basal cell proliferation, especially in areas of epithelial damage. Additionally, there is marked intrapulmonary airways damage, with extensive epithelial denudation. Of interest, in the trachea and large airways 80% of the proliferating cells were $KRT5^{+}$ (i.e. were basal cells) whilst 70% of proliferating cells in the small airways lacked KRT5 expression [(5)]. However, the impact of SARS-CoV-2 infection on ciliary function in patients recovering from COVID-19 has not been explored. Here, we report the effects of COVID-19 on nasal ciliated epithelium from 6 hospital inpatients from whom biopsies were taken within one month of diagnosis.

Our study was limited to six subjects, between 2- and 31-days following COVID-19 diagnosis (Table 1). Ethical approval was obtained through the Living Airway Biobank (REC reference 19/NW/0171). Nasal brushing was used to obtain respiratory epithelial tissue.

The methodology for ciliary functional analysis has been previously described [(6)]. All samples were studied in a containment level 3 research laboratory. Briefly, high-speed video microscopy images were analysed by observing, where possible, 10 ciliated cells in 10 different regions of the movie sequence, which represented 10 respiratory epithelial cells. Ciliary beat frequency (CBF) was calculated from recordings made at 500 frames/second (fps) and replayed at 30 fps. By counting the time (fps) it took for one cilium to complete 5 beat cycles the CBF was calculated using the formula 500/fps elapsed x 5 ciliary beat cycles counted. Ciliary beat pattern was determined as being normal or dyskinetic viewed from the side profile of the sample [(7)]. The percentage of each beat pattern category was calculated using the total number of readings as 100%.

Transmission electron microscopy (TEM): epithelial samples from nasal brushing were fixed in 4% glutaraldehyde in Sorensen's phosphate buffer (pH 7.4) and processed as previously described [(7)].

Of the six patients studied one had evidence of near normal ciliary function. No cilia were found in patient 2, and all of the cilia of the remaining four patients had a dyskinetic beat pattern with very high levels of dyskinesia in patient 1, 4, and 6 (Table 1). On electron microscopy, no ciliated cells were found in samples from patients 1, 2 and 6, , although no recognisable cellular

Table 1. Patient information, ciliary beat frequency & ciliary dyskinesia.

Patients	Gender	Age	Days from COVID-19 diagnosis	Taste and Smell	Smoker	Co-morbidities	CBF (Hz) (mean, 95% CI)	Dyskinesia (%)
Patient 1	F	57	15	Affected	N	Asthma	10 (7.4 – 12.1)	100
Patient 2	M	80	6		N	Asthma, Hypertension, Heart Failure	No cilia	No cilia
Patient 3	M	87	11	Affected	N	Liver abscess + biliary sepsis	10.4 (9.5 – 12.3)	38
Patient 4	M	62	2		N	None	4.7 (0 – 8.4)	100
Patient 5	F	62	31	Affected	N	None	9.4 (8.4 – 10.2)	77
Patient 6	F	60	7		N	Type 2 diabetes	6.8 (0 – 14.9)	100

* We have previously reported Ciliary Beat Frequency (CBF) of healthy adults using our system (11.5Hz: 95% CI 10.3-12.7 Hz) [7].

structures were seen in patient 6 EM samples. A marked reduction in the percentage of ciliated cells in patient 4 (6.8%) and patient 5 (23.1%) were seen in EM samples. Normal levels of ciliation were seen in patient 3 (69%)(Figure 1C), In patient 1, where SARS-CoV-2 virus was shown in association with abnormal epithelial protrusions, densely packed intermediate filaments were seen throughout the sample with a lack of normal cellular ultrastructure (Figure 1A).

We found that the majority of the six patients hospitalised with COVID-19 infection studied had marked nasal epithelial damage and ciliary dysfunction. Extensive abnormalities of the ciliated epithelium were seen in five of the six COVID-19 patients with a marked epithelial disruption and reduced ciliation in four. No cilia were observed in one patient where, although tissue was obtained, no recognisable cellular structures such as nuclei or mitochondria were seen.

In three patients all of the cilia observed were beating in a dyskinetic fashion. In a further patient, 77% of cilia displayed an abnormal ciliary beat pattern. In one patient, despite all cilia beating dyskinetically, ciliary beat frequency remained within our previously published normal range [6], a dissociation we have previously reported following coronavirus infection of healthy individuals [6] and in primary ciliary dyskinesia [8]. This suggests that following infection, a normal ciliary beat frequency cannot be relied upon to exclude abnormal ciliary function. In one patient, who was being treated in hospital for biliary sepsis and subsequently became infected with COVID-19, only 38% of cilia had an abnormal beat pattern whilst the majority of cilia had a normal ciliary beat pattern and frequency. Whilst other viral, bacterial and other insults can also affect ciliary function, it is highly likely that the findings are secondary to COVID-19 infection.

Despite our findings of extensive nasal respiratory epithelial damage, a study by Stavem and colleagues [9] in patients hospitalised with COVID-19 reports only 38% of patients recalled symptoms of a runny nose whilst nearly 70% suffered from loss or disturbance of smell, with animal studies showing extensive olfactory epithelial damage [10]. Of interest, in a previous study where healthy volunteers were nasally inoculated with a coronavirus (HCoV), 25% reported no symptoms despite extensive ciliated epithelial damage [6].

Our results suggest that epithelial damage, reduced ciliation and ciliary dysfunction are features of the majority of hospitalised COVID-19 patients. It will be important to determine epithelial recovery, as this has been shown to be prolonged in the nasal epithelium following RSV positive bronchiolitis, taking 13-17 weeks to return to normal [11]. However, our results cannot be extrapolated to patients with COVID-19 who are asymptomatic or have mild disease, and further studies are underway to investigate this. It will be important therefore, to determine the time course of functional ciliated epithelial recovery in COVID-19, as epithelial dysfunction and ciliary dysfunction may contribute to a patient's respiratory disease.

Abbreviations

ACE2 = Angiotensin-converting enzyme 2; CBF = Ciliary beat frequency; COVID-19 = Coronavirus disease 2019; FPS = Frames per second; HCoV = Human coronavirus; KRT5 = Keratin 5; RSV = Respiratory syncytial virus; SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2; TEM = Transmission electron microscopy

Acknowledgements

This research is supported by the NIHR GOSH BRC and by GOSH Children's Charity. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Depart-

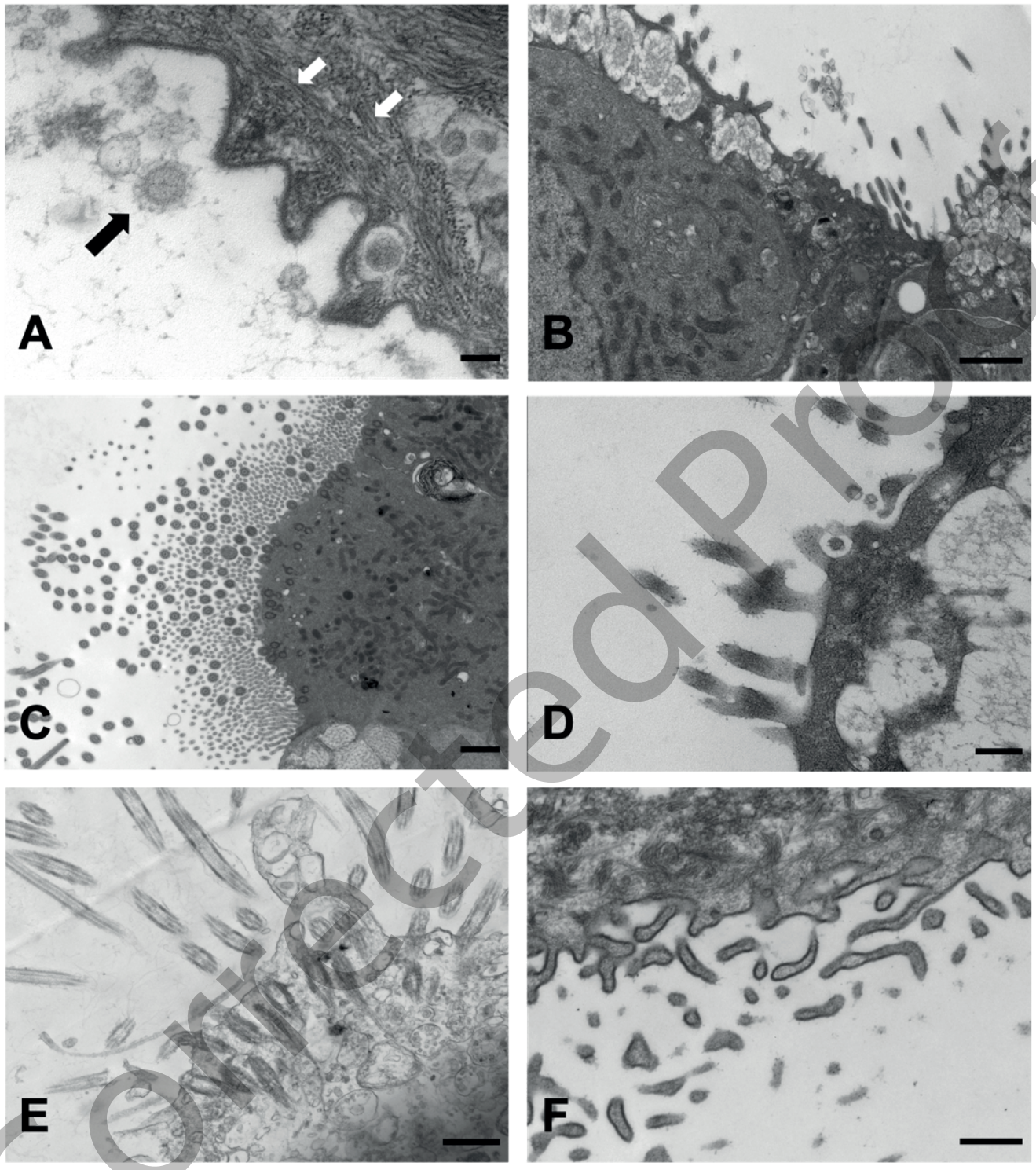

Figure 1. Transmission electron microscopy findings in patients recovering from COVID-19. Illustrative electron micrographs and general description of findings: A) Patient 1 - Normal cilia not seen. The epithelium also lacks normally formed microvilli and normal cellular ultrastructure. Large amounts of intermediate filaments are seen within the tissue near the surface (white arrows). A SARS-CoV-2 virus (black arrow) is seen close to the epithelial tissue. Similar epithelial edges were seen in the sample. Scale bar = 100nm; B) Patient 2 - Epithelial tissues lacks cilia. Normal microvilli seen and a much greater amount of mucus was present than in healthy ciliated epithelium. Surface epithelium: 87.8% unciliated; 12.2% mucus cells. Scale bar = 800nm; C) Patient 3 - Healthy ciliated epithelium with densely packed microvilli. Surface epithelium 69% ciliated; 27.6% unciliated; 2.4% mucus cells. Scale bar = 1µm; D) Patient 4 - Lack of cilia with normal microvilli and enhanced amount of mucus present compared to healthy respiratory epithelium. Surface epithelium: 6.8% ciliated; 79.7% unciliated; 13.6 % mucus cells. Scale bar = 200nm; E) Patient 5 - Dead ciliated cell. Surface epithelium: 23.1% ciliated (mostly dead); 61.5% unciliated; 15.3% mucus cells. Scale bar = 600nm; F) Patient 6 - No recognizable cellular structures seen on epithelial edges. Scale bar = 400nm.

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Authorship contribution

DDHL, DC, and CO designed the study. YS contributed to the patient sample and data collection. DDHL, DC, RAH, NW, VC, AR, and TH performed experiments and contributed to data analysis and interpretation. DDHL, DC, and CO wrote the manuscript draft. REH, TM, PR, CMS, and CO reviewed and edited the manuscript.

Conflict of interest

All authors declare that they do not have potential conflicts of interest.

Funding

DC and DDHL are funded by Great Ormond Street Hospital Charity and Sparks National Funding Call 2017/2018 (V4818 - Restoring ciliary function in primary cilia dyskinesia). REH is a Wellcome Trust Sir Henry Wellcome Fellow (WT209199/Z/17/Z).

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