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Early View

Research letter

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Radiological-pathological signatures of patients with COVID-19-related pneumomediastinum: is there a role for sonic-hedgehog and Wnt5a pathways?

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Research letter

To the Editor:

Pneumomediastinum (PM) is a rare complication of acute respiratory distress syndrome (ARDS) when air leaks into the mediastinum. An increased PM incidence, of up to 5-13%, was reported during the COVID-19 pandemic [1,2] and even occurred spontaneously without a history of mechanical ventilation [3], similarly to the previous SARS-1 [4]. Almost half of the 30 consecutive COVID-19 patients who had prolonged invasive mechanical ventilation had full-thickness tracheal lesions and PM [5]. Although pronation and high PEEP levels were presumed to be the putative causes of PM [5], the mechanism of this COVID-19 complication remains unknown. We hypothesize that sonic hedgehog (SHH) and Wnt5a signaling, crucial pathways in tracheal morphogenesis, and repair/regeneration of cartilage lesions in adulthood [6,7], could play a role in PM-related COVID-19 tracheal lesions.
This study assessed the PM characteristics in hospitalised COVID-19 patients through a retrospective analysis of a prospectively collected COVID-19 database to identify the clinical-radiological and pathological signatures associated to severe SARS-CoV-2 virus infection.

Methods
This observational study was carried out from 10th March, 2020 to 28th February, 2021 at the University Hospital of Trieste and was approved by the local Ethical Committee (CEUR#2020-Os-148). The patient or next of kin provided written informed consent.
Inclusion criteria were *(a)* hospitalized patients with pneumonia positive for COVID-19 confirmed by a positive reverse-transcription (RT)-PCR analysis from nasopharyngeal or oropharyngeal swabs [8], *(b)* age 18 years or older and *(c)* chest CT scan confirmed pneumomediastinum . Volumetric unenhanced CT images were acquired (Revolution EVO GE Healthcare, Chalfont St Giles, UK) and reconstructed with a slice thickness of 1 mm and lung sharp kernels. All CT images were evaluated by three radiologists aware of the COVID-19 status, but blinded to other clinical information. Clinical data were obtained from the local electronic clinical records. Three pathologists blinded to patient identification performed a masked pathological study on the autoptic trachea specimens from patients who died of PM. The patients' hematoxylin and eosin slides were reviewed separately and anonymously. Immunohistochemistry (IHC) was performed on formalin-fixed and paraffin-embedded (4-Gm-thick) sections using antibodies directed against Sonic-Hedgehog, SHH (Abcam, clone EP1190Y, rabbit monoclonal anti-human, 1:100) and Wnt5a (TermoFisher, clone 3D10, mouse anti-human monoclonal, 1:200). IHC assays were assessed by a semi-quantitative ranking score, ranging from 0, for no labelling, to 4 for intense labeling, in the slices of cartilage ring remodelling [9].
Statistical analysis were performed using the software R (rel. 4.0.2). The Gaussian distribution of continuous variables was assessed by the Shapiro-Wilk test. The study population characteristics were assessed by mean ± SD or median (IQR), as appropriate. Continuous variables were compared by t-tests or Mann–Whitney tests.

Results

A total of 1,098 consecutive patients with COVID-19 pneumonia were reviewed and 54/1,098 (4.9%) had an unenhanced CT scan of the thorax and met the eligibility criteria. There was a 44.6% PM-associated mortality rate, making it a severe complication of the disease, whilst the study population mortality rate of hospitalized patients was 22.8% (p-value=0.002).

The median age of patients with PM was 73 years (IQR, 64-77), 40 (74.1%) were males (29.2% smokers), and most patients (n=43, 81.1%) had comorbidities (cardiopathy 52.8%, hypertension 50.9%, obesity 31.4%, diabetes 30.2%, cancer 17.3%, COPD 16.6%, immune depression 3.2%). Pneumomediastinum alone was detected in 40 patients (74%), while the remaining 14 patients had associated pneumothorax (10 mono-lateral partial, 3 mono-lateral complete, 1 bi-lateral pneumothorax). The prevalent lung parenchyma CT scan pattern was ground glass in 20 patients (37%), alveolar filling 11 patients (20.4%), mixed 10 patients (18.5%), crazy paving 7 (13%), and reticular/fibrotic pattern 6 patients (11.1%). Spontaneous PM occurred in 4 patients (7.4%), 1 patient (1.8%) received HFNC while had PM, 30 patients (55.6%) NIV/CPAP, and 19 patients (35.2%) were invasively ventilated.

We observed no statistical significant differences regarding clinical characteristics, co-morbidity, and CT scan features between deceased and surviving patients. Only invasive mechanical ventilation was significantly associated with death.

There was a 9 day median duration (IQR, 3-13) of mechanical ventilation from intubation to PM. An autopsy was carried out on the 23 patients with PM who died and 100% of them had tracheal/large airway lesions. A control group of 8 patients who died of non-COVID-19 ARDS were also pathologically studied. Autopsy showed that most of the COVID-19 PM patients had full thickness tracheal/large airway lesions (29 patients, 53.7%), two had tracheal fistula (Fig. 1A) while the remaining 16 PM patients had tracheal mucosal ulcers and/or cartilage lesions. All the autopsies showed peculiar findings of the tracheal/airways cartilage, specifically fibrous-hyaline degeneration (Fig. 1B and 1C), that were not present in the trachea and main bronchi of control ARDS subjects. The immunohistochemistry staining evidenced a strong Wnt5a expression (labelling score 3.69 ± 0.55) and a weak SHH expression (labelling score 2.21 ± 0.90) in the cartilage cells of the tracheal/bronchial rings of the COVID-19 patients with PM. Controls were negative for both Wnt5a and SHH for cartilage regeneration biomarkers (labelling score 0.25 ± 0.46 for both SHH and Wnt5a, p=0.0001).

Discussion

Both spontaneous and mechanical ventilation-related pneumomediastinum was observed in our series of COVID-19 patients. Moreover, pneumomediastinum was more isolated than associated to pneumothorax. Autopsy of patients with PM evidenced diffuse tracheal/large airway cartilage lesions with fibrous-hyaline degeneration. Although some of these lesions could represent an iatrogenic effect of mechanical ventilation and a superimposed bacterial infection, no association with intubation or bacterial pneumonia was observed. Therefore, they more likely represent an intrinsic feature of COVID-19.

Other authors already observed an increased PM incidence which was not explainable by barotrauma or high transpulmonary pressure. Our findings are consistent with cartilage remodelling

during COVID-19 and ARDS, without a complete post-injury regenerative process [10]. Indeed, two major pathways of chrondrocyte regeneration had a significantly different expression in our autoptic samples, i.e., aberrantly expressed Wnt5a and weakly expressed SHH in the injured cartilage tissue. This is quite different to the usual molecular crosstalk involving Wnt5a and SHH during tracheal development and adult tissue regeneration [7]. In our PM patients tracheal and large airways were frequently injured and an abnormal regenerative process with cartilage tissue remodelling occurred as a pathologic signature, absent in non-COVID ARDS controls. The fibrous-hyaline degeneration of the tracheal rings seen in our autoptic series is an original observation which has not been previously described in COVID-19 patients.

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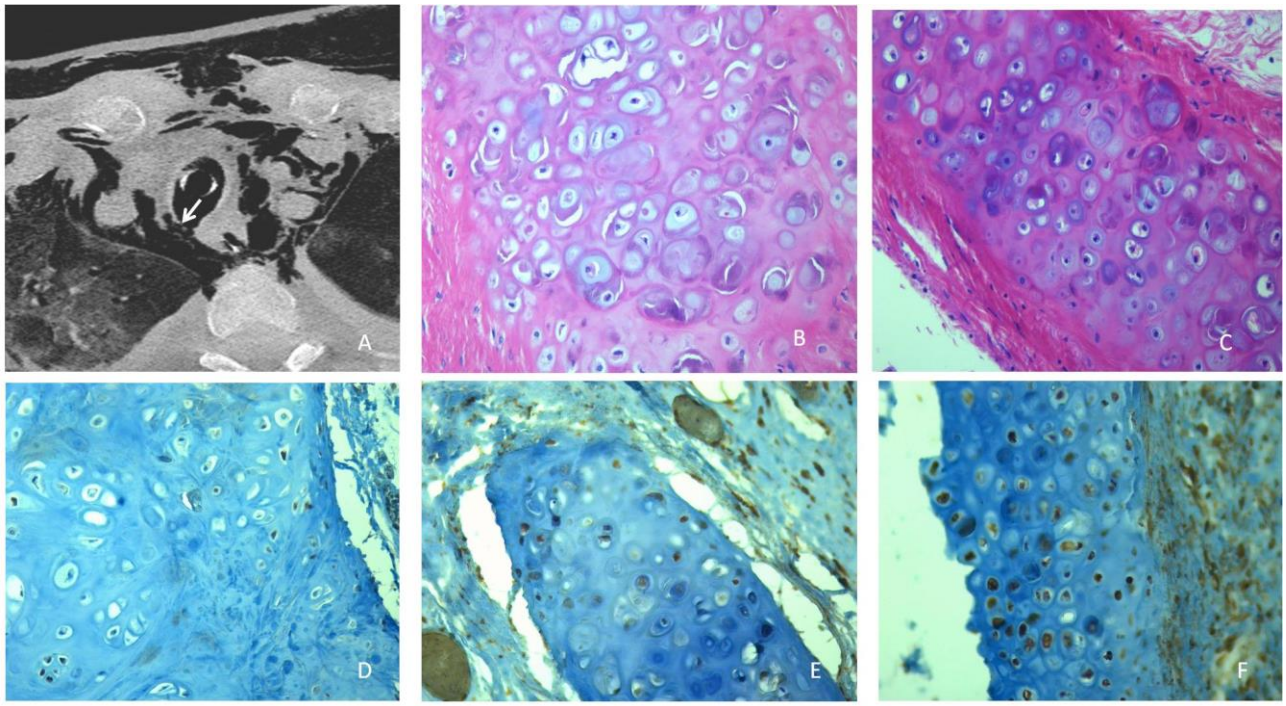

Figure 1. Legend

1A= Axial chest CT scan showing a full-thickness tracheal lesion with fistula; 1B= tracheal cartilage micro-cracks in patient with COVID-19 and pneumomediastinum (HE x40); 1C= basophilic cartilage matrix and intracellular fracture with chondrocyte apoptosis (HE x20); 1D= control non-COVID ARDS with absent Sonic Hedgehog (IHC x20); 1E= weak positive SHH tracheal chondrocytes in a COVID-19 patient (IHC x20); 1F= strong expression of Wnt5a in the same patient (IHC x20).