Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Atypical lymphocytes in bronchoalveolar lavage fluid from patients with COVID-19 ARDS

We read with interest the recent paper by El Jamal and coworkers published in *Pathology – Research & Practice* [1] which reported that conspicuous atypical plasmacytoid lymphocytes are present in peripheral blood smears from patients with coronavirus disease 2019 (COVID-19), which is in line with other recent studies [2,3]. Indeed, many of the patients in these reports were critically ill and required intensive care therapy, and in one of the studies, similar cells were noted bronchoalveolar lavage fluid (BALF) [2]. The exact nature and role of these atypical lymphocytes in the pathophysiology of COVID-19 nevertheless remains a *terra incognita*.

In a recent study [4], we investigated the compartmental cellular and humoral immuno-phenotype in blood and BALF of patients with COVID-19 acute respiratory distress syndrome (ARDS). The study encompassed four mechanically ventilated patients admitted to the ICU with moderate to severe ARDS (for patient characteristics, see Table 1), and was approved by the Regional Ethics Committee of Copenhagen (H-20023159), the Knowledge Centre for Data Review of Copenhagen (P-2020-399) and registered at clinicaltrials.gov (NCT04354584), and oral and written informed proxy consent was obtained from the next of kin as well as the patient's general practitioner. They patients were all were included <72 h after intubation, and none were treated with corticosteroids, antivirals or immunomodulatory drugs off-protocol. In terms of lymphocyte counts and function, we found notable changes in T cells, including a reduced thymic output with a depleted and hyperactivated T cell population, of which the latter was more prominent in the lungs than in blood [4]. Here, we report additional cytomorphological analyses of BALF from this study.

Five cytospins each containing one to three drops of BALF were made from each of the four patients. Two cytospins were stained with May-Grünwald-Giemsa (MGG), and three were left for immunohistochemical staining for CD3 and CD138, which are surface markers used to identify T cells and plasma cells, respectively. MGG revealed the presence of the same atypical plasmacytoid lymphocytes previously described in blood smears in the BALF of all four patients (Fig. 1A). Hence, the atypical lymphocytes contained a basophilic cytoplasm with a perinuclear clearing and in some cells small intracytoplasmic vacuoles were observed. The nucleus was enlarged with an oval to irregular shape and contained clumped chromatin and prominent nucleoli. Immunohistochemical subtype characterisation was somewhat difficult due to the relatively sparse amounts of cell in the cell-block and the cells were not all well conserved. However, they did express CD3 (Fig. 1B) while only relatively few were found weakly positive for CD138 (Fig. 1C). Thus, this cell population likely represents highly reactive virocyte-like T cells rather than plasma cells. The variation in the count of this cell type in each sample was significant ranging from only a few cells to 30 % of the leukocytes, but was nonetheless was a consistent finding in all four patients.

Similar but not identical cell-types have previously been described in case reports of influenza A/H1N1 pneumonia complicated by ARDS [5], but atypical lymphocytes with this morphology is not typical of other know pneumonia-causing viral diseases [1,2], and has not, to the best of our knowledge, previously been described in BALF of any other disease. Potentially, this cytomorphological pattern may thus be pathognomonic for COVID-19.

It remains to be elucidated whether the presence of atypical virocyte T cells within a depleted and hyperactivated T cell population in the lungs are important to the progression of lung parenchymal damage in COVID-19, or if they alternatively merely comprise an epiphenomenon to the severe pulmonary hyperinflammation, which is often coined a 'cytokine storm' [6]. In any event, we speculate that the relatively high occurrence of these cells in the lungs compared to that of blood (<10 % of lymphocytes in blood [2]) reflects that their terminal differentiation occurs in the lungs, and that the atypical lymphocytes reported in peripheral blood mainly reflects a 'spill-over' from the alveolar compartment. Future studies should perform an in-depth characterisation of the virocyte-like lymphocytes by additional immuno-histochemical analysis and cell sorting, as well as functional studies, so that it may be determined if the circulating atypical lymphocytes are indeed derived from the lungs, whether they are pathognomonic for COVID-19, and how they potentially contribute to the severity and progression of disease.

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Author contributions

RMGB, AR, and RRP developed the protocol and designed the study. AR and RRP collected the data. SBR performed the pathological analyses. RMGB prepared the first draft of the manuscript and completed all revisions. RMGB and RRP handled funding and supervision. All authors provided critical input at all stages, interpreted the data and were involved in drafting and editing of the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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Table 1
Patient characteristics. Patient characteristics have also been published elsewhere [4]. The laboratory data are provided for the last blood sample obtained prior to the bronchoscopy procedure. *SARS-CoV-2 was detected by polymerase chain reaction, while the other reported pathogens were identified by BioFire® FilmArray® Pneumonia Panel. Abbreviations: BALF, bronchoalveolar lavage fluid; HT, hypertension; T2D, type 2 diabetes.

Patient No.	1	2	3	4
Age (yr.)	40	65	72	75
Sex	M	F	M	M
Coexisting disorder	None	Asthma	T2D, HT	HT
Duration of symptoms before admission to hospital (days)	4	11	4	3
Duration from hospital admission to bronchoscopy procedure at ICU (days)	6	2	8	15
Laboratory data				
Leukocyte count (E^9/L)	3.8	9.8	12.3	10.8
Neutrophil count (E^9/L)	3.1	8.6	9.9	8.0
Lymphocyte count (E^9/L)	0.34	0.65	0.92	0.94
C-reactive protein (mg/L)	340	320	250	30
Procalcitonin (μg/L)	25.6	0.58	20.4	0.73
Microbiology* (BALF)	SARS-CoV-2, *Sphingomonas spp.*	SARS-CoV-2, *S. aureus*, *N. meningitidis*, *S. agalactiae*	SARS-CoV-2	SARS-CoV-2
Outcome	Survived	Died	Died	Survived

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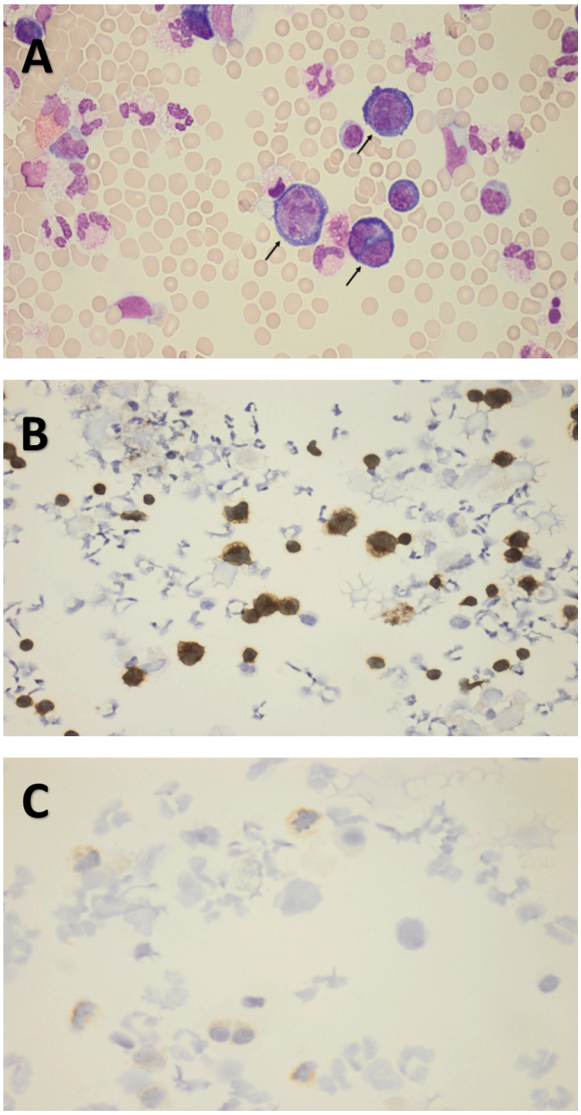

Fig. 1. Atypical lymphocytes in bronchoalveolar lavage fluid from a patient with COVID-19 ARDS. A. May-Grünwald-Giemsa staining (x40) showing conspicuous atypical plasmacytoid lymphocytes (arrows). B: Immunehistochemical staining for CD3. C: Immunehistochemical staining for CD138.

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