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Original Contribution

Diffuse interstitial pneumonia-like/macrophage activation syndrome-like changes in patients with COVID-19 correlate with length of illness

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ABSTRACT

Objectives: Assess the pathologic changes in the lungs of COVID-19 decedents and correlate these changes with demographic data, clinical course, therapies, and duration of illness.
Methods: Lungs of 12 consecutive COVID-19 decedents consented for autopsy were evaluated for gross and histopathologic abnormalities. A complete Ghon "en block" dissection was performed on all cases; lung weights and gross characteristics recorded. Immunohistochemical studies were performed to characterize lymphocytic infiltrates and to assess SARS-CoV-2 capsid protein.
Results: Two distinct patterns of pulmonary involvement were identified. Three of 12 cases demonstrated a predominance of acute alveolar damage (DAD) while 9 of 12 cases demonstrated a marked increase in intra-alveolar macrophages in a fashion resembling desquamative interstitial pneumonia or macrophage activation syndrome (DIP/MAS). Two patterns were correlated solely with a statistically significant difference in the duration of illness. The group exhibiting DAD had duration of illness of 5.7 days while the group with DIP/MAS had duration of illness of 21.5 days (*t*-test $p = 0.014$).
Conclusions: The pulmonary pathology of COVID-19 patients demonstrates a biphasic pattern, an acute phase demonstrating DAD changes while the patients with a more prolonged course exhibit a different pattern that resembles DIP/MAS-like pattern. The potential mechanisms and clinical significance are discussed.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel viral pathogen that principally infects the respiratory system. The current pandemic designated as COVID-19 has demonstrated that the highest mortality is occasioned by lower respiratory tract compromise. Severe cases are characterized by progressive respiratory failure. Clinically, patients who experience severe illness develop respiratory symptoms such as labored breathing, often with fever, followed by a sudden and rapid decline in their ability to oxygenate blood [1]. Radiologically patients develop peripheral lung ground glass opacities on computed tomography (CT) [2].

The pathophysiology of the disease is still poorly understood, but the severity appears to depend on the development of a cytokine storm that overwhelms human adaptive immunity, with high levels of IL6, IL10, TNFα, and D-dimer and CD4 and CD8 cytopenia [3]. Although cytokine release likely plays a role in COVID-19, several differences have been noted that set the disease somewhat apart from other causes of acute respiratory distress syndrome (ARDS). Lymphopenia and thrombosis are not hallmarks of cytokine storm-associated diseases but are seen in COVID-19 and several cytokines such as interleukin-6 and inflammatory mediators such as ferritin appear to be less elevated in COVID-19 compared to classical cytokine storm-associated diseases [4].

Several reports in the literature have outlined the pulmonary changes including diffuse alveolar damage with superimposed bacterial infection, intra-alveolar fibrin deposition or acute fibrinous and

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Table 1
Patient demographics and results summary.

Patient	Age	Gender	Race/ ethnicity	Date of autopsy	Length of hospital stay (days)	Time on a ventilator (days)	DIP-MAS-like	Diffuse alveolar damage	Lympho-cytic infiltrate	Pneumonia	Thrombotic micro-angiopathy	PE	Pulmonary hemorrhage	Thrombi	Fibroblastic foci	Comorbidities
1	57	Female	Hispanic	4/27/ 2020	2.71	2.53	No	Yes	No	Yes	Yes	No	Yes	Yes	No	Systemic hypertension; Diabetes mellitus type 1; atherosclerosis – coronary; Asthma; Obesity
2	57	Female	White	4/29/ 2020	22.63	19.1	Yes	No	No	No	No	No	No	Yes	Yes	Systemic hypertension; Diabetes mellitus type 2; Atherosclerosis – Aortoiliac; Asthma; Obesity
3	81	Male	White	4/30/ 2020	20.94	7.15	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes	Systemic hypertension; Atherosclerosis - intracranial, thoracic aorta; Asthma
4	28	Male	Black	5/14/ 2020	14.60	8.92	Yes	No	Yes	No	No	No	No	No	Yes	Systemic hypertension; Diabetes mellitus type 2; Asthma; Obesity; OSA; Depression; CVA
5	57	Female	Black	5/27/ 2020	35.73	13.17	Yes	No	Yes	No	No	Yes	No	No	No	Systemic hypertension; Atherosclerosis – coronary; Asthma; Obesity; Hypothyroidism
6	81	Female	White	5/28/ 2020	5.70	N/A	No	No	No	Yes	No	No	No	No	No	Systemic hypertension; Diabetes mellitus type 2; Atherosclerosis – coronary; Obesity; ESRD (on HD); Pulmonary HTN; PAD
7	66	Female	White	6/6/ 2020	13.49	N/A	Yes	No	Yes	No	No	No	Yes	No	Noo	Systemic hypertension; Atherosclerosis - intracranial; Obesity; OSA; Depression
8	81	Male	Black	6/8/ 2020	8.72	4.78	No	Yes	No	No	Yes	Yes	Yes	Yes	No	Systemic hypertension; Diabetes mellitus; atherosclerosis – coronary; Asthma; Obesity; OSA; Prior pulmonary embolism on anticoagulation; Lewy body dementia; Primary adrenal insufficiency; Chronic kidney disease; Chronic obstructive pulmonary disease; Gout
9	76	Female	White	6/23/ 2020	11.77	8.15	Yes	Yes	No	No	Yes	No	No	No	Yes	Systemic hypertension; Obesity; Cardiomyopathy; Chronic kidney disease
10	91	Male	Black	6/28/ 2020	10.66	N/A	Yes	Yes	No	No	No	No	No	No	Yes	Systemic hypertension; Diabetes; hypothyroidism; chronic kidney disease; anemia of chronic disease
11	63	Female	Hispanic	6/28/ 2020	9.62	5.83	Yes	Yes	No	No	No	No	No	No	No	Systemic hypertension; Diabetes mellitus type 2; Atherosclerosis – aorta; Asthma; hypothyroidism; bilateral lung transplant due to idiopathic; pulmonary fibrosis; chronic lymphopenia; MGUS; chronic kidney disease; chronic normocytic anemia
12	71	Female	White	7/15/ 2020	16.04	N/A	Yes	No	Yes	Yes	No	No	No	No	No	Systemic hypertension; Atherosclerosis – intracranial; Neurofibromatosis type 1; Hx of GIST; HX spindle cell hemangioma; Cognitive impairment/developmental delay

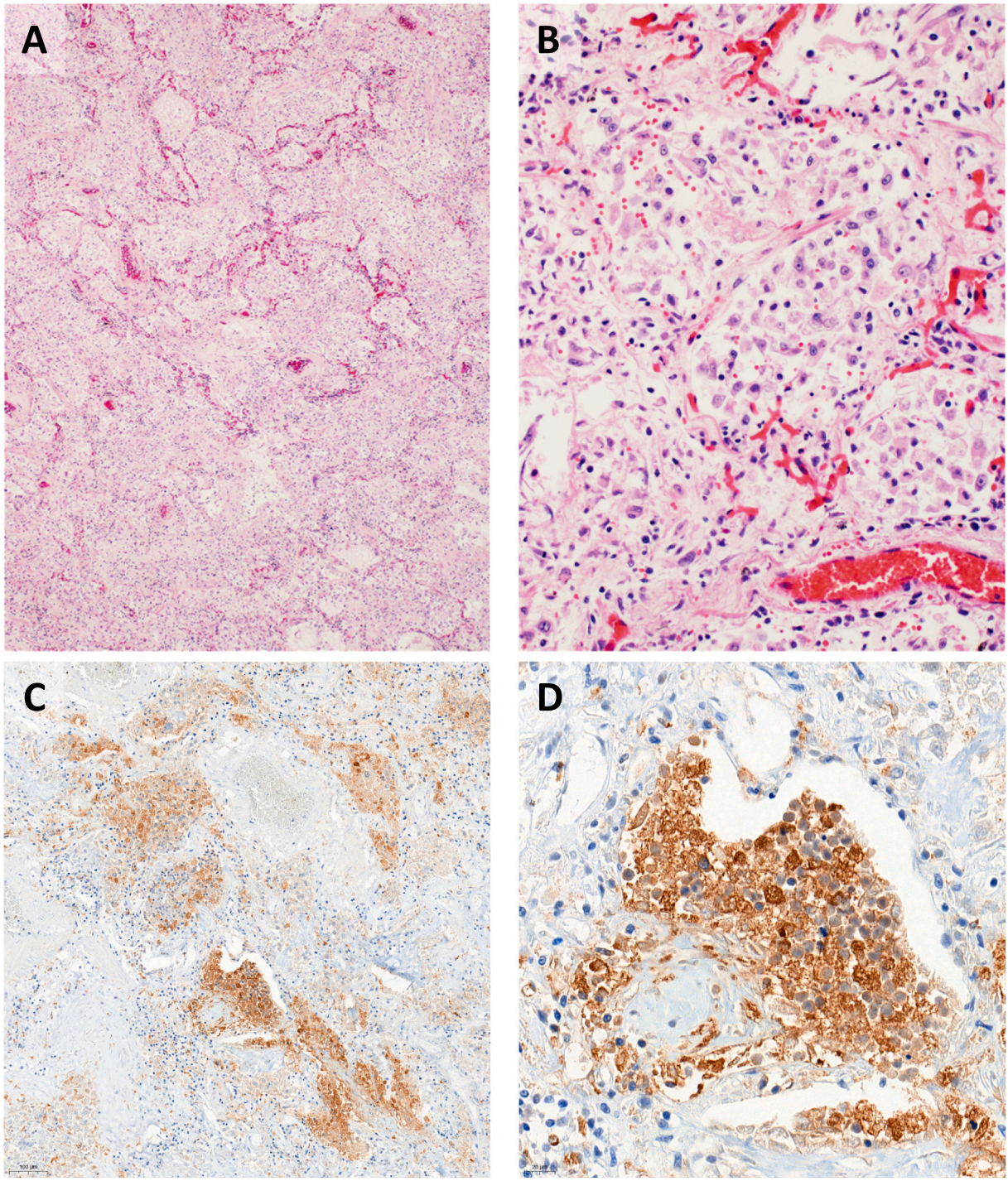

Fig. 1. Desquamative interstitial pneumonia (DIP)-like/Macrophage Activation Syndrome (MAS)-like changes. Images of lung tissue from a patient who died 26 days after hospitalization exhibiting the characteristic increase in intra alveolar macrophages seen in the chronic phase of the disease. Images are of hematoxylin and eosin (H&E) stained lung sections at low, 40× (***A***) and high, 400× magnification (***B***). Visualization of macrophages by immunohistochemistry for CD68 also at low, 40× **(*C*)** and high, 400× ***(D)*** magnification.

organizing pneumonia (AFOP) and thrombotic microangiopathy (TMA) [5,6]. Mapping of the entire spectrum of histologic changes seen in COVID-19 patients is expected to improve our understanding of the disease and may lead to new treatment strategies. In this work, we report the pulmonary observations in twelve COVID-19 autopsies, including the finding of frequent Diffuse Interstitial Pneumonia-like/Macrophage Activation Syndrome-Like changes that are expected to have bearing on the pathophysiology of the process.

2. Methods

Twelve consecutive decedents with a diagnosis of COVID-19, in which autopsy consent was obtained and verified, were selected for this report. The autopsies were carried out between 6 and 79 h after death. Demographic data on the decedents is reported in Table 1. A complete autopsy was performed utilizing the “en-block” or Ghon method and all organs weighed and examined in their entirety. Tissue specimens were

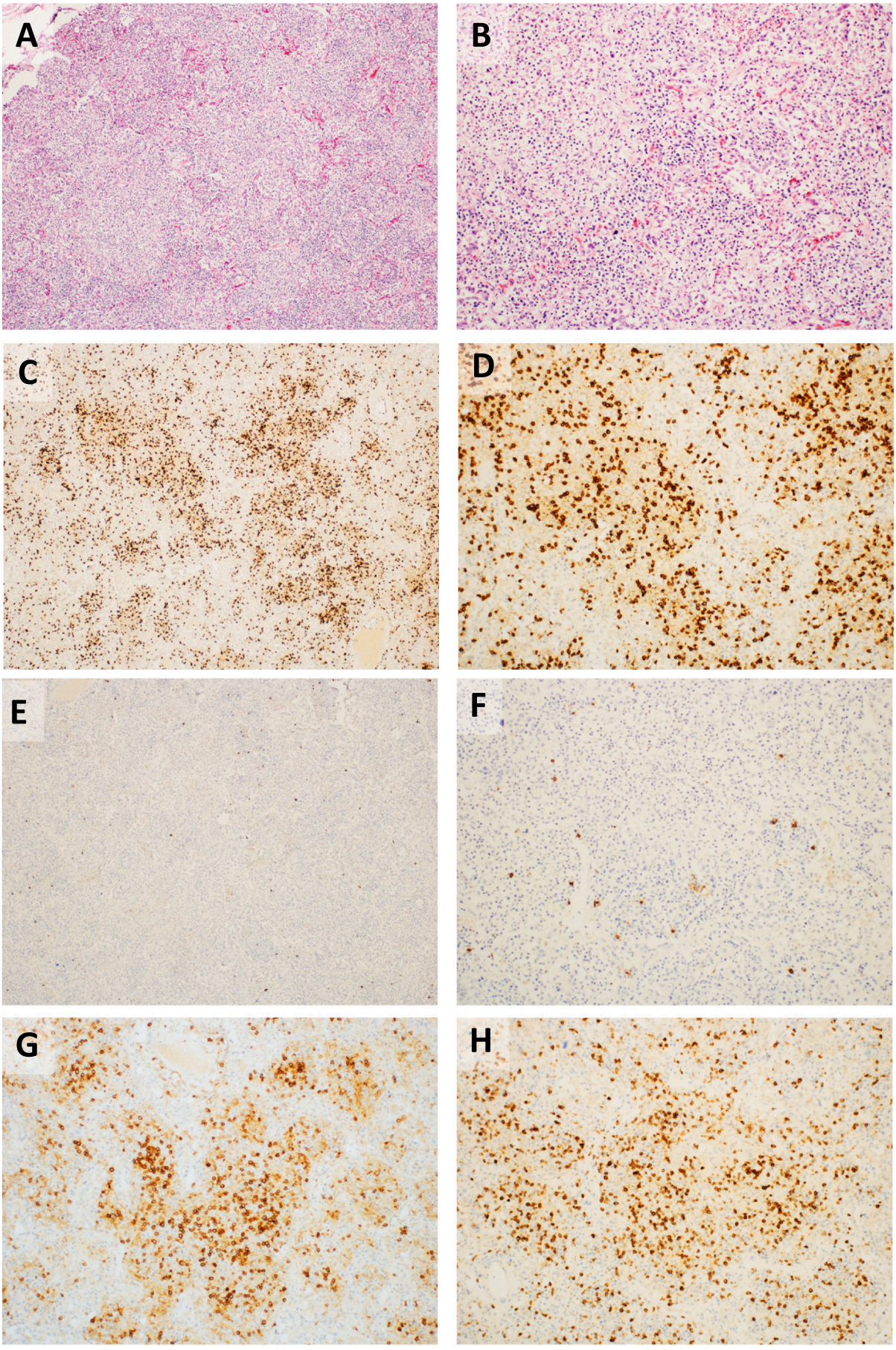

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Fig. 2. Secondary pattern of the chronic phase of COVID-19 involvement. Lung tissue from a patient who died 29 days after hospitalization exhibiting the secondary chronic pattern that includes increased intra alveolar macrophages as well as an interstitial and intra alveolar infiltrate of small lymphocytes. Images are shown of hematoxylin and eosin (H&E) stained lung sections at low, 40× (***A***) or intermediate, 200× magnification (***B***). Immunohistochemical visualization of CD3 positive T cells at a low, 40× (***C***), and intermediate 200 X magnification (***D***). Immunohistochemical visualization of CD20 positive B cells can be seen at a low, 40× magnification (***E***) and at an intermediate 200× magnification (***F***). CD4-positive T-lymphocytes (***G***) and CD8-positive T-lymphocytes (***H***) at intermediate, 200× magnification showing an relative equal distribution.

routinely fixed in 10% buffered formaldehyde for 48 h as recommended and then processed for histopathological evaluation. Routine H&E stained microscopic sections were prepared from most major organs including sections of all lobes of the lung. In addition, microscopic sections were prepared from selected blocks and subjected to immunohistochemistry.

Immunohistochemistry for SARS-CoV-2 Nucleocapsid protein was performed using an affinity-purified polyclonal rabbit antibody (ProSci cross-validated protocol as described) [7]. Briefly, deparaffinized histological sections of lung tissues were incubated with anti-SARS-CoV-2 Nucleocapsid protein antibody (affinity-purified rabbit IgG; ProSci, Poway, CA; Cat# 9099; 0.04 μg/ml), followed by peroxidase blocking reagent, HRP-conjugated secondary antibody-polymer, substrate chromogen, and Hematoxylin counterstain.

Immunohistochemistry for CD68, CD3, CD20, CD4, and CD8 was performed in a Bond III automated system, Leica Biosystems (Buffalo Grove, IL) using ready to use antibodies as per manufacturers specifications.

3. Statistical analysis

A two-sided Student's *t*-test was used to compare mean time from hospitalization to death between patients with or without DIP/MAS-like lung histopathology.

4. Results

The demographic data, selected clinical information, and the pulmonary histological observations in twelve consecutive autopsies of decedents with COVID-19 are summarized in Table 1. Of the twelve, nine decedents exhibited pulmonary accumulation of innumerable histiocytes or macrophages that filled alveoli and terminal bronchioles. These changes are morphologically similar to the changes seen in desquamative interstitial pneumonia (DIP) or in macrophage activation syndrome (MAS) (Fig. 1). Five of the cases displayed this change as the predominant pattern whereas in four this pattern was more subtle and focal. The histiocytes frequently contained foamy cytoplasm and lacked the typical pigment seen in cigarette smokers with DIP. These macrophages also failed to exhibit hemophagocytosis, a feature almost universally found in MAS.

The three patients in whom the DIP/MAS-like pattern was not observed displayed the more commonly described lesions seen in COVID-19 affected patients, including diffuse alveolar damage with hyaline membrane formation and intra alveolar fibrin deposition. Two patients had organizing pneumonia and two patients had thrombotic microangiopathy.

In 3 cases where the DIP/MAS-like pattern was predominant and one where the finding was more focal an abundant infiltrate of small lymphocytes was also present. Immunohistochemical characterization of the lymphoid infiltrate revealed virtually all of the cells to be CD3 positive with a fairly even distribution of CD4 and CD8 positive lymphocytes; the cases were virtually devoid of CD20 positive B-cells (Fig. 2). In the cases with this DIP/MAS-like pattern, the lungs either lacked or were only slightly involved by diffuse alveolar damage in the form of hyaline membrane formation or intra-alveolar fibrin deposition. The three cases where hyaline membranes still remained with DIP/MAS-like changes were also the three cases with the shortest duration from diagnosis to death in the group of nine. Among the 12 decedents, thrombotic microangiopathy and superimposed bronchopneumonia were seen in two cases each, while pulmonary emboli and pulmonary hemorrhage were seen in one case each.

Immunohistochemical (IHC) studies to detect SARS-CoV-2 virus revealed detection of viral nucleocapsid protein in lungs of patients who died within 10 days of hospitalization, whereas the virus protein was generally cleared in lungs of patient patients who died after more prolonged duration of the disease. Viral protein levels in lungs by IHC correlated well with viral mRNA levels extracted from adjacent histological sections as measured by qRT-PCR (data not shown). Representative images of SARS-CoV-2 Nucleocapsid protein expression in lungs of patients who died during acute phase (<10 days after hospitalization) and chronic phase (>20 days after hospitalization) are shown in (Fig. 3***)***. During acute phase, high viral protein levels were detected in desquamated pneumocytes as validated by pan-cytokeratin staining (data not shown), as well as in debris from lysed cells and in hyaline membranes.

Evaluation of the clinical findings revealed that the only correlation with the DIP/MAS-like change was time from hospitalization to demise. The patients with DIP-like findings had a longer time from hospitalization to demise than the patients without the finding. Specifically, patients with a predominant or focal DIP-like pattern had an average time from hospitalization to death of 21.5 days, significantly longer than the corresponding mean 5.7 day period in patients without the finding (*t*-test $p = 0.014$). The temporal association is supported by the clearance of the SARS-CoV-2 virus as determined by Nucleocapsid protein IHC in the cases of DIP-like change whereas the virus remained detectable in the three cases without the DIP-like change corresponding to demise earlier in the decease process. There was no association between the DIP/MAS-like changes and any given medication that these patients received nor with any treatment including intubation, convalescent plasma or dexamethasone treatment.

5. Comment

Our observation of a DIP/MAS-like condition in the lungs of COVID-19 decedents is interesting and its temporal association from time of hospitalization to demise is potentially of great significance. The patients with this finding had worsening hypoxia despite maximal life support measures and the absence of superimposed bronchopneumonia or pulmonary hemorrhage in most seven of the nine cases, and absence of pulmonary embolism in all but one case. The lungs lacked significant interstitial fibrosis or emphysema, making the DIP/MAS-like changes the likely cause of the patients' extreme inability to oxygenate their blood. Similar findings have recently been reported in a cohort of 16 patients where the presence immune cells, including macrophages and T-cells, were correlated with length of hospitalization [8]. In that study, the authors also correlated the finding of intra-alveolar macrophages with low quantities of interferon stimulated genes (ISG-low) when compared to patients who died at an earlier stage that had high levels of interferon stimulated genes. They also found decreased viral loads in ISG-low cases when compared to ISG-high cases. In the present cohort we also observed a correlation between viral Nucleocapsid protein expression and length of survival and were unable to detect viral protein or RNA in the DIP/MAS-like group.

The presence of macrophages in the lung of COVID-19 patients are likely necessary in order to phagocytize the cellular debris caused by the infected dying and desquamating pneumocytes and carry off the debris out of the lungs. This process is known as efferocytosis. Efferocytosis is

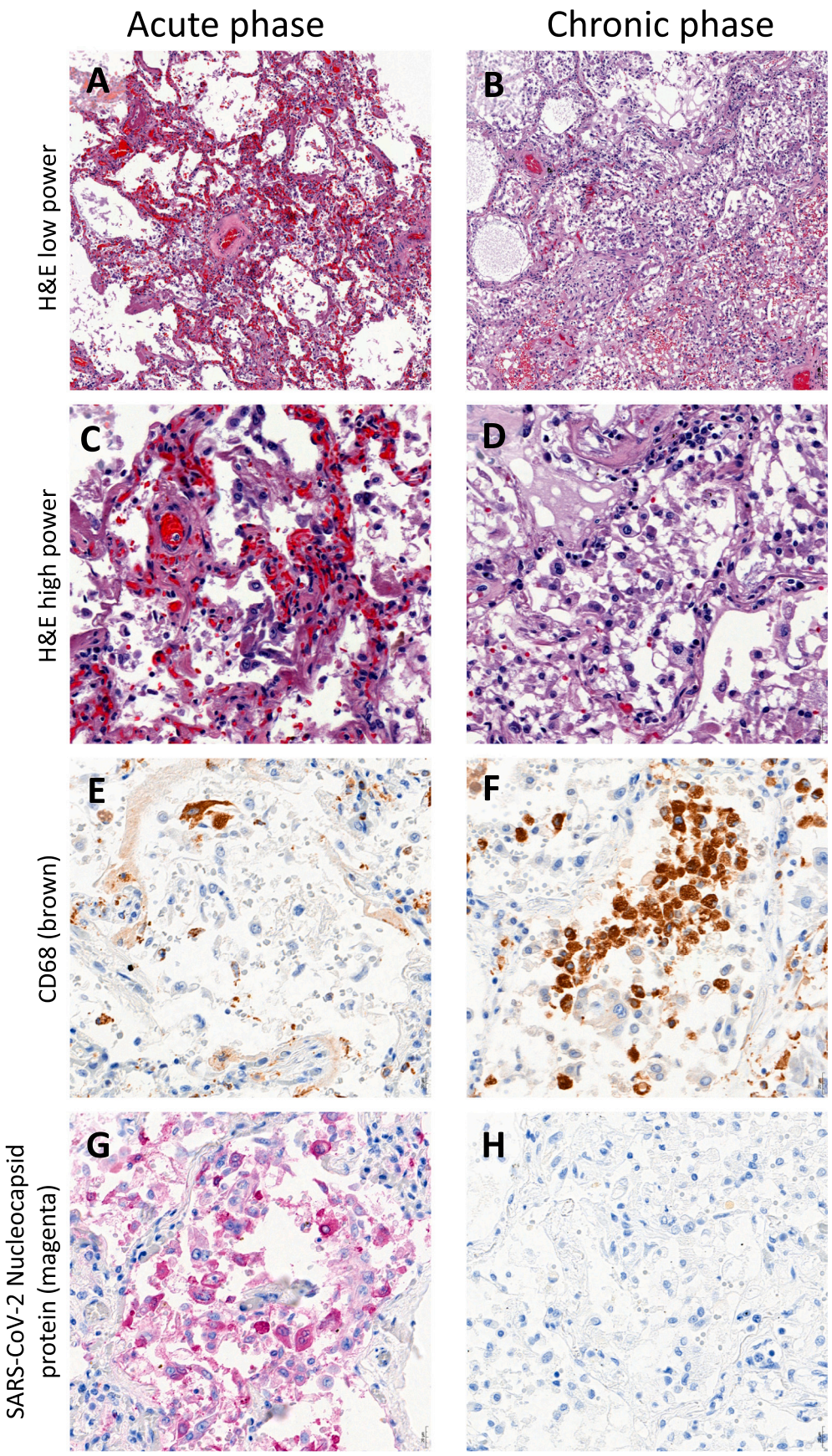

Fig. 3. Presence of SARS-CoV-2 virus protein in lungs during acute phase but not chronic phase of the disease. Images of lungs from the acute phase (left column) are from a patient who died ~3 days after hospitalization and from the chronic phase (right column) are from a patient who died >20 days after hospitalization. Images of hematoxylin and eosin (H&E) stained lung sections at low, 40× magnification (***A,B***) and at an intermediate, 200× magnification (***C,D***). Visualization of macrophages by immunohistochemistry of CD68 (brown; ***E,F***) at 200× magnification. Visualization of SARS-CoV-2 by immunohistochemistry of Nucleocapsid protein (magenta; ***G,H***) at 200× magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

necessary for appropriate return of the lung to normal structure and function following pneumocyte death and acute alveolar damage. The massive accumulation of macrophages seen in these COVID-19 decedents in later stage disease would suggest that the normal process of clearance is either overwhelmed by the quantity of cell debris or could be suggestive of defective efferocytosis [9].

Alternatively, the histopathologic findings described in these nine decedents could represent a variant of (MAS), also known as secondary hemophagocytic lymphohistiocytosis. MAS-like conditions have been reported in COVID-19 patients [10]. Classic MAS is seen in patients with chronic rheumatic diseases, mainly adult onset Still's disease (AOSD) and is associated with a poor outcome. Although these conditions may appear similar at first glance, the absence of hemophagocytosis in COVID-19 patients would suggest a different process and likely a different mechanism. The abundance of macrophages within the alveoli in patients with COVID19 with ARDS may contribute to their dismal prognosis in it of itself, as they do in MAS patients, despite the effective clearing of the virus in the upper and lower airways by the immune system.

The presence of alveolar macrophages is one of histopathologic manifestation of an acute lung injury. However, the extent of the phenomenon in these autopsies is unusual. It is also reminiscent to the changes seen in post-obstructive pneumonia. In the COVID-19 settings the DIP/MAS-like condition could indicate occlusion of the small airways caused by inflammation. Our data suggest that the patients with this type of lung injury endure longer, compared to the patients with classic DAD, but ultimately succumb to the disease.

The presence of widespread DIP/MAS-like changes in the lungs of COVID-19 patients is poorly understood. Most studies in COVID-19 patients suggest that fatalities are triggered by an inflammatory cytokine storm leading to CD4+ and CD8+ lymphocyte dysfunction which blocks adaptive immunity. It has been postulated that high levels of cytokines, such as IL6, IL10, and TNFα may be mediated by alveolar macrophages in these patients [11]. As lymphocytes are depleted of ACE2 receptors necessary to COVID-19 cell entry, lymphopenia and lymphocyte impaired survival and functional exhaustion could be ascribed in part to macrophage dysfunction [12]. Since accumulation of intraalveolar macrophages appears to represent a late event in COVID-19 disease, the deregulation of macrophages is unlikely to be induced by direct viral infection. SARS-CoV-2 immunoreactivity has been previously demonstrated in alveolar macrophages [13] and viral particles have also been demonstrated within macrophages in human hearts [14] raising the possibility that the virus may be causing direct macrophage dysfunction. In our series, viral nucleocapsid proteins was identified predominantly in pneumocytes and uncommonly in respiratory epithelium. Credible staining was absent in alveolar macrophages, a fact that again fails to support direct viral damage to the macrophages.

In conclusion, we have identified a DIP/MAS-like lesion in COVID-19 patients that appears to be a late development in the disease. The paucity of other changes in these decedents point to this being a possible if not likely cause of death for these patients. Efforts to investigate the possible mechanisms for this development should be vigorously pursued and strategies to combat it developed.

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Declaration of competing interest

None.

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