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Lesions in the lungs of fatal corona virus disease Covid-19

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ABSTRACT

The corona virus outbreak in Wuhan, China, at the end of 2019 has rapidly evolved into a pandemic which is still virulent in many countries. An infection with SARS-CoV-2 can lead to corona virus disease (Covid-19). This paper presents an overview of the knowledge gained so far with regard to histopathological lung lesions in fatal courses of Covid-19. The main findings were diffuse alveolar damage and micro-angiopathies. These included the development of hyaline membranes, thrombi, endothelial inflammation, haemorrhages and angiogenesis. Overall, the vessel lesions seemed to be more lethal than the diffuse alveolar damage. There was obvious hyperreactivity and hyperinflammation of the cellular immune system. An expanded T-cell memory may explain the increased risk of a severe course in the elderly.

The Severe Acute Respiratory Syndrome Corona Virus2 (SARS-CoV-2) has so far caused more than twenty million infections and resulted in over one million deaths(WHO Covid-19 statistics). In the meantime some pathological studies have been conducted and recently summarized (Mohanty et al., 2020). This short review reports on some of the findings from autopsies and morphological patterns complemented with results of our own investigations.

1. Diffuse alveolar damage

Diffuse alveolar damage, as seen in acute respiratory distress syndrome, was the most frequent histological diagnosis and occurred in overlapping stages and was accompanied with interstitial and endothelial/intimal inflammation (Fig. 1AE). Acute inflammation is prevalent during the first weeks coming along with oedema, pneumocyte hyperplasia, infiltrates and fibrinous exudate(Carsana et al., 2020). From the third week onwards, remodelling or repair may follow (Sweeney and McAuley, 2016). In the study published by Carsana et al., 38 lungs from patients, who had died of Covid19, were investigated (Carsana et al., 2020). This cohort was made up of 33 men and 5 women with a mean age of 69. Thirty-one of them had known co-morbidities, for instance diabetes or hypertonia. Different pulmonary pathological alterations were observed in all or in most cases in this study: capillary congestion (all), pneumocyte necrosis or apoptosis (all), AEC II hyperplasia (all), oedema (37 cases) and hyaline membranes (33 cases). The capillary congestion and the endothelial cell damage might be responsible for subsequent ischaemia. Other changes such as myofibroblast reactions, intraalveolar fibrin deposits, alveolar wall thickening, squamous metaplasia and syncytial giant cells were also observed. In nearly 90 % of the patients platelet-fibrin thrombi were documented and were also observed in own investigations (Fig. 1FG).

AEC II were found to be full of virus particles, detected by means of electron microscopy (Carsana et al., 2020). This is an interesting finding, however the mechanism remains unclear. During experimental lung infection in mice, pseudomonades were detected in AEC II (Schmiedl et al., 2010). The occurrence of intracellular viruses or bacilli might either be the consequence of an infection or invasion or an active engulfment by the AECII or both. Thus, AEC II might play an important role in the pathophysiology of viral and bacterial pneumonia. The receptor-binding domain (RBD) of the SARS-CoV-2 club-like, spike protein binds to the cell membrane protein Angiotensin Converting Enzyme-2 (ACE-2), which is expressed by epithelial cells of the alveoli, bronchi and tracheobronchial glands (Ackermann et al., 2020; Lan et al., 2020; Ren et al., 2006). There it converts Angiotensin I into Angiotensin II

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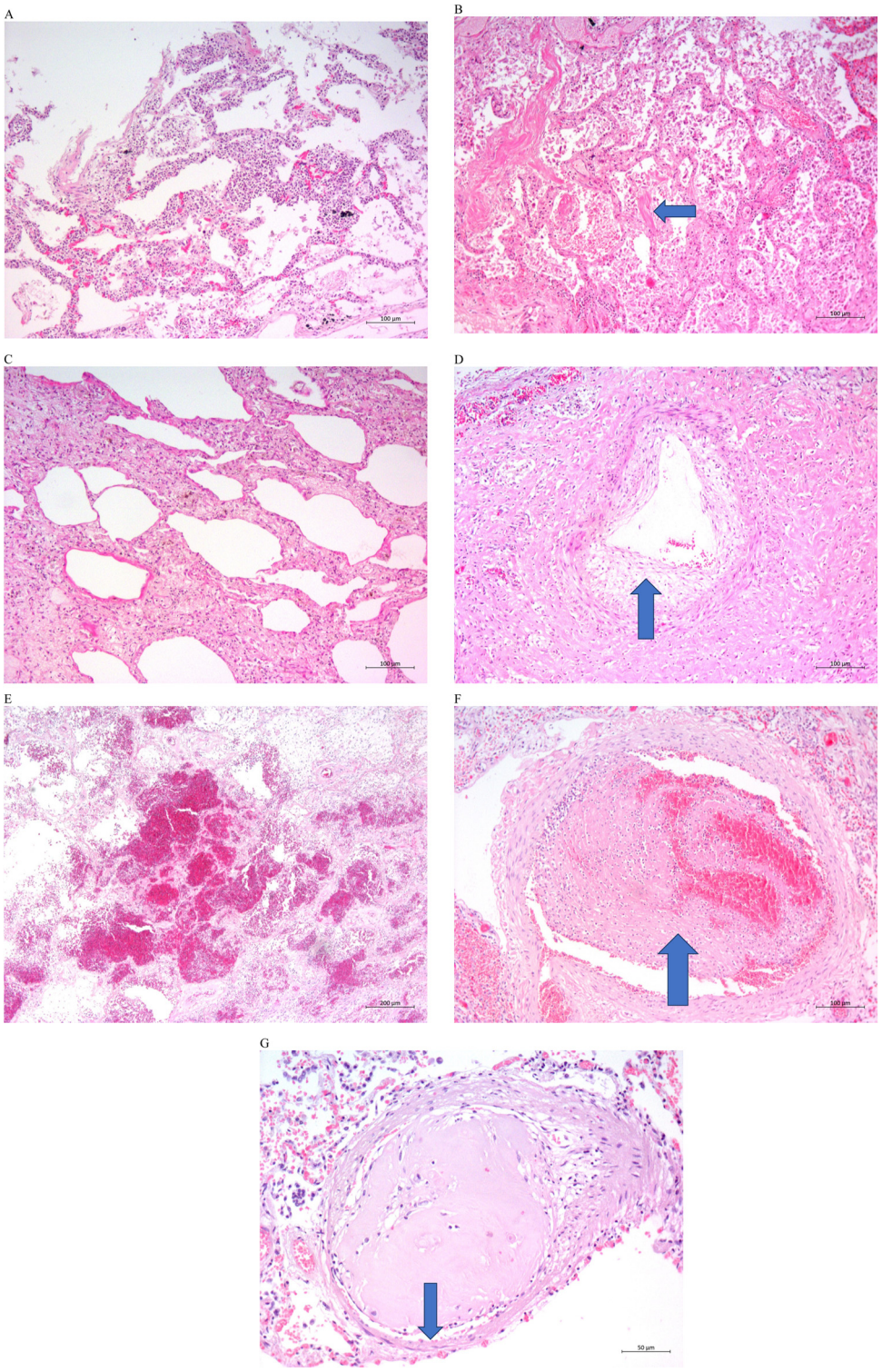

Fig. 1. This figure presents examples of lung histopathology from patients who died of Covid-19 (H&E staining).
A Covid-19 lung disease: Lymphocytic infiltration of alveolar septae, indicating endothelitis (HE, original magnification x10)
B Covid-19 lung disease: Diffuse alveolar damage with desquamative interstitial pneumonia pattern and alveolar fibrin deposits (arrow) (HE, original magnification x10)
C Covid-19 lung disease: Nonspecific interstitial pneumonia (NSIP)-like pattern with diffuse cellular thickening of alveolar septae (HE, original magnification x10)
D Covid-19 lung disease: Post inflammatory intimal hyperplasia of a muscular pulmonary artery, diffuse alveolar damage and atelectatic obliteration (arrow) (HE, original magnification x10)
E Covid-19 lung disease: Acute alveolar haemorrhage and diffuse alveolar damage (HE, original magnification x20)
F Covid-19 lung disease: Thrombus and loss of endothelium (arrow) in a mid-sized muscular pulmonary artery (HE, original magnification x10)
G Covid-19 lung disease: Fibrin-rich thrombus and loss endothelium (arrow) in a small muscular pulmonary artery (HE, original magnification x20)

through limited proteolysis. The RBD-ACE-2-complex leads to the transcription of genetic information of the host cells. The virus was also documented in macrophages, albeit in lower amounts than in pneumocytes (Lan et al., 2020).

The immune system plays an important role during a SARS-CoV-2 infection. The viral infection and expression of foreign proteins initiates immune cascades and inflammation. Most obvious is the release of chemokines by the T cells, which in turn attracts neutrophil granulocytes, leading to inflammation and tissue damage. The acute inflammatory stage of the diffuse alveolar damage might be the histopathological correlate of the immune response. Increased numbers of alveolar macrophages (24 cases) and interstitial lymphocyte aggregates (31 cases) were found (Carsana et al., 2020). Moreover, Fox et al. described that $CD4^+$ lymphocytes showed the tendency to cluster around smaller vessels, while $CD8^+$ lymphocytes preferentially accumulated around larger vessels and bronchioles (Fox et al., 2020). A crucial question remains with regard to the infiltration of lymphocytes: Do lymphocytes combat or support the propagation of the virus? Ackermann et al. documented the presence of ACE-2 positive lymphocytes in Covid-19 patients, but not in the control group (Ackermann et al., 2020). Infected ACE-2 positive lymphocytes could provide one possible explanation for the propagation of the virus towards other organs such as the kidneys. (Micro)perivascular lymphocytic infiltration has often been found during pulmonary inflammation in the (micro)perivascular space of lung vessels - and especially arteries - as described in detail by Pabst (Guntheroth et al., 1982; Pabst, 2004; Pabst and Tschernig, 2002, 2006; Schmiedl et al., 2005; Shiang et al., 2009; Singh et al., 2005; Tschernig et al., 2008). Lymphocytic perivascular infiltration was also found in lung specimens from autopsies of Covid-19 patients (Ackermann et al., 2020; Wagner et al., 2020). One hypothesis is that cytokines released into the alveoli and the interstitial tissue, are drained by lymphoid vessels which line the pulmonary vessels. A high concentration of pro-inflammatory mediators in perivascular lymphoid vessels could then lead to oedema and cellular infiltration within the perivascular space. It remains unclear whether perivascular inflammation is beneficial in lung injury and inflammation or the opposite.

2. Microangiopathies

The death rate was 45% higher in Covid-19 patients with a microangiopathy- induced pulmonary embolism than in patients without pulmonary embolism (Fox et al., 2020; Liao et al., 2020). Ackermann et al. compared findings in acute respiratory distress syndrome of Covid-19 and influenza (Ackermann et al., 2020). In this study, groups, similar in terms of age, gender and the severity of the disease, were classified. Methods such as histology and immunohistochemistry, electron microscopy, micro-computed tomography, corrosion specimens and gene expression analyses were employed. In both groups, diffuse alveolar damage with associated perivascular T cell infiltration was observed. In addition, various vessel pathologies were found mainly in patients of the Covid-19 group. These might be explained by the necrosis of endothelial cells infected with SARS-CoV-2 and subsequent endothelitis. The main features of the lesions were detachment of the basal membrane, disruption of intercellular junctions and swelling of endothelial cells (Ackermann et al., 2020). Microthrombi were nine times more frequent in the alveolar capillaries of the Covid19 group than in those of the influenza group. An additional interesting finding was the observation of intussusceptive angiogenesis, the term fora process in which one vessel splits into two vessels (Burri and Djonov, 2002; Burri et al., 2004). Pillar-like figures, characteristic of this process, were distinguishable in electron microscopic images. The number of newly-formed vessels in the Covid-19 group was about 3 times higher than in the influenza group. Thus, angiogenesis might occupy essential room within the alveolar walls and thereby worsen lung function. From all genes previously associated with angiogenesis, 69 were differentially regulated in the Covid-19 group but only 26 in the influenza group (Ackermann et al., 2020). The authors hypothesised that the described angiogenesis might be a consequence of endothelial inflammation as well as of the high number of thrombi.

Important roommates of endothelial cells are pericytes, which are probably also responsible for intussusceptive angiogenesis (Burel-Vandenbos et al., 2020; Cardot-Leccia et al., 2020). A loss of pericytes due to an infection with SARS-CoV-2 might cause the proliferation of endothelial cells and induce intussusceptive angiogenesis. This hypothesis was supported by increased ACE2 expression in pericytes compared to endothelial cells (Cardot-Leccia et al., 2020). Thus, a loss of pericytes infected with SARS-CoV-2 might be a fitting explanation for the pathogenesis of microangiopathies.

Fox et al. found haemorrhages in 9 out of 10 examined lungs of patients aged 44–78 years, who had died of Covid-19. Interestingly, this author discovered several platelets producing CD61 immunopositive megakaryocytes inside the alveolar capillaries. They are considered to be responsible for contributing to the generation of the platelets/fibrin thrombi, which occur in high numbers. Moreover, authors of that study and others pointed out that megakaryocytes are known to express antiviral genes during an above average infection, e.g. interferon-induced transmembrane protein 3 (Campbell et al., 2019).

A strong immune reaction might favour the pathogenesis of diffuse alveolar damage and, in consequence, lead to a severe progression of the disease. The latter idea is in contrast to the prevalent view that a younger patient's immune system is stronger than that of an elderly person, as most of the severe courses are patients with an average age of between 60 and 80. However, the comprehensive memory of the immune system in elderly persons might play an important role in the development of tissue damage in Covid-19. The strong suspicion that at least two further cell membrane proteins apart from the spike protein of SARS-CoV-2 can trigger an immune reaction consolidates this concept as this does, in fact, occur in other pathogens (Babel et al., 2020; Thieme et al., 2020). In general, many patients who experienced severe courses of the disease suffered from co-morbidities as well as from the effects of artificial respiration during their treatment. Indeed, many artefacts or confounders may influence the histopathology and the overall number of samples is still limited (Hariri and Hardin, 2020). This paper only refers to fatal disease outcomes, however it should be kept in mind that the majority of patients are confronted with a mild course of the disease and with long-term consequences.

In conclusion, Covid-19 causes lesions of the lung tissue with an alveolar component (diffuse alveolar damage, hyaline membranes) and with a vascular component (endothelitis, thrombi, angiogenesis, haemorrhages), both with serious consequences for the outcome of the disease. The high fatality rate in infected patients over the age of 70 might be influenced by the expanded T cell memory in that population.

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