A review of post infectious pulmonary fibrosis in the era of COVID-19 and potential treatment options

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ABSTRACT

On March 11, 2020, the World Health Organization (WHO) released a statement characterizing COVID-19 as a pandemic that has, as of October 2020, caused almost 36 million confirmed global cases and over 1 million deaths. One of the long-term complications suggested by researchers is fibrosis. It has been hypothesized that the combination of ongoing pulmonary injury caused by COVID-19 and the inability to promptly repair damage results in interstitial matrix widening and eventual compression and destruction of alveoli and capillaries. Here we focus on pathogenesis, risk factors, different infectious causes of fibrosis along with COVID-19, and potential treatment options that might reduce its effects.

Key words: COVID-19, pulmonary fibrosis, mechanism, treatment

INTRODUCTION

On March 11, 2020, the World Health Organization (WHO) released a statement characterizing COVID-19 as a pandemic,[1] a disease outbreak that has spread over multiple countries and continents.[2] As of October 2020, there have been almost 36 million confirmed global cases and over 1 million deaths caused by this disease.[3] COVID-19, or Coronavirus Disease 2019, is caused by SARS-CoV-2, a virus in the Coronaviridae family of viruses. These viruses usually cause upper respiratory tract illnesses, like the common cold. Most viruses in this family do not infect humans, and most of the seven coronaviruses known to infect humans cause only mild to moderate disease. However, three viruses can cause a more serious and potentially fatal disease.[4] SARS coronavirus emerged in 2002 and was found to cause severe acute respiratory syndrome (SARS); MERS (Middle East respiratory syndrome) coronavirus emerged in 2012 and continues to cause localized outbreaks around the world. The third deadly coronavirus is SARS-CoV-2. Because this disease is caused by a virus that belongs to a family of previously studied viruses, we can infer or hypothesize potential outcomes of the infection before we have the concrete studies needed to support theories. This paper will discuss the potential for lung fibrosis as a long-term complication in patients who have been infected by SARS-CoV-2.

MECHANISM OF PULMONARY FIBROSIS

Idiopathic pulmonary fibrosis (IPF) is the most common interstitial lung disease (ILD), a group of pulmonary disorders characterized by inflammatory changes in the alveoli that results in irreversible fibrosis and, subsequently, impaired lung function. It is likely caused by excessive replication of type II pneumocytes in response to alveolar damage or micro-injuries, caused by several potential factors. In normal lungs, damage to type I pneumocytes that line the alveoli causes a sequence of changes starting with proliferation and differentiation of type II alveolar cells and stem cells and type II epithelial cells that restore the alveolar epithelium through activation of coagulation, angiogenesis, fibroblast activation and migration,

collagen synthesis and proper alignment. These processes are performed through the different actions of the chemokines, such as TGF-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). In normal lungs, type II pneumocytes stimulate fibroblasts in the interstitium to become myofibroblasts in response to TGF-beta. These myofibroblasts produce reticular and elastic fibers, which are then secreted outside of the cell into the interstitium. The reticular fibers are responsible for structural support, while the elastic fibers give the lung its elasticity. The myofibroblasts then undergo apoptosis, and the cycle stops.[5,6,8]

In fibrotic lungs, the alveolar repair cycle becomes irreversible. Micro injuries responsible for repetitive injuries to the type I pneumocytes render the type II pneumocytes dysfunctional, leading to functionally impaired lungs.[7] Repetitive injuries to the lung trigger protein overexpression and imbalance between the cellular demand of protein synthesis and protein folding and maturation in the endoplasmic reticulum (ER), leading to misfolding of the proteins known as the "ER stress."[7] Consequently, another pathway becomes activated, leading to not only inhibition of protein translation and targeting them for degradation but also causing apoptosis of the cells when the stress persists. This response is known as the unfolded protein response (UPR). This response, along with resulting in activation of intracellular apoptotic pathways, also induces the production of profibrotic mediators, such as TGF-β1, PDGF, CXCL12 (C-X-C motif chemokine 12), and CCL2 (chemokine C-C motif ligand 2).[5] Among those, TGF-β1 is the most important factor that leads to alveolar apoptosis, epithelial-mesenchymal transition (EMT), epithelial cell migration, increased replication and activation of myofibroblasts in the interstitium, and production of VEGF and CTGF connective-tissue growth factor), including other profibrotic and pro-angiogenic mediators responsible for several other pathways.[8] The proinflammatory chemokines and cytokines produced by the damaged type I pneumocytes lead to several reactions in the lungs, including increased vascular permeability, new vessel formation, and endothelial cell proliferation, including endothelial progenitor cells (EPCs), which is a normal response to any injury. Malli et al. demonstrated that IPF patients have significantly reduced numbers of EPCs, resulting in failure of reendothelialization, formation of dysfunctional alveolar-capillary barriers, stimulating profibrotic response, and resultant augmentation of VEGF. This growth factor leads to abnormalities in vessel function, such as increased vascularization in the area surrounding the fibrotic foci, subsequently facilitating fibrogenesis.[9,10] Consequently, the overproduction of collagen (e.g., reticular and elastic fibers) and thickening of the interstitium between the alveoli and the capillaries leads to ventilation-perfusion mismatch within the lungs and decreases lung compliance due to the excess collagen fibers.[11] The restricted lung expansion causes changes in pulmonary function tests consistent with restrictive lung disease with decreased total lung capacity (TLC), forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV1).[12] The process of fibrosis is thought to be irreversible and progressive. The combination of ongoing pulmonary injury and the inability to promptly repair damage results in an interstitial matrix widening and eventual compression and destruction of alveoli and capillaries. This process substantially contributes to the respiratory failure that can occur within 3–7 years.[13]

RISK FACTORS

Several risk factors have been implicated in the pathogenesis of IPF, including physiological aging leading to resistance to apoptosis along with increased fibrotic responses after injury with environmental stimuli, such as cigarette smoking and metal dust, agriculture, and farming, livestock, wood dust, stone, silica, sand, and microbial agents, such as pathogenic bacterial and viral infections.[5] However, in recent years, genetic susceptibility and changes in gene expression of individuals developing IPF to environmental triggers have also become apparent. Rare genetic mutations have been found in the genes of IPF in adults, including surfactant protein C (SFTPC) and A2 (SFTPA2), leading to protein misfolding in the ER of type II pneumocytes and enhanced ER stress respectively.[14]

Researchers have also found rare variants in genes regulating telomerase and telomerase-associated proteins, such as TERT and TERC that led

to telomere shortening.[15] In studies on changes in gene expression, transcriptional and translational changes in the profile of mRNA expression have also been found in the lungs of the IPF patients. Nance et al. in 2014 identified 873 differentially expressed genes in lung biopsies of eight IPF patients compared with seven controls, and 675 showed alternative splicing events in genes coding for periostin (POSTN) and collagen (COL6A3).[16] Depianto et al. and Bridges et al. used microarrays to identify differential expression of 2940 and 781 genes, including genes encoding for growth factors, collagens, proteinases, and cytokines.[17,18] Bridges et al. found the Twist1 gene, a gene with a protective function against apoptosis, was the most upregulated in IPF lungs. Studies were also done to assess gene expression that leads to acute exacerbations of IPF. Konishi et al. identified 579 differentially expressed genes associated with acute exacerbations with cyclin A2 (CCNA2) and α-defensins being the most upregulated.[19] Lung biopsies of patients with progressive IPF also showed differential expression of 243 transcripts, including C-C Motif Chemokine Ligand 2 (CCL2) and SFTPA1 when compared with lung biopsies from stable IPF patients.[20]

In addition to changes in gene expression, epigenetic alterations, such as DNA methylation, microRNA dysregulation, and histone modifications, have also been implicated in changes in the IPF lung.[21] Yang et al. established a significant association between DNA methylation and gene expression by recognizing 2130 genome-wide differentiated methylated regions in 94 tissues of IPF patients.[22] Cigarette smoking and aging have been identified as the main effectors of epigenetic modifications in IPF patients since both are responsible for IPF and DNA methylation.[23,24] In recent studies, infections, especially by viruses (Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus, hepatitis B virus, human papillomavirus, HIV) and intracellular bacteria (*Helicobacter pylori*, chlamydia, mycobacteria, and salmonella) have also been found to be responsible for significant DNA methylation, histone methylation, histone acetylation, and other epigenetic changes.[25] However, clinical studies have not been done to identify the relationship between virus-induced epigenetic changes and the development of IPF.

INFECTIOUS CAUSES OF FIBROSIS

The first association of viral infection and IPF was made in 1953 when many patients had a viral-type prodrome preceding respiratory symptoms.[26] One of the first viruses that was suspected to be associated with IPF was hepatitis C virus (HCV), which causes fibrosis in the liver. However, in 1992, Japanese researchers Ueda et al. discovered a higher prevalence of HCV markers in IPF patients (28.8%) than in control subjects.[27] In 2008, Arase et al. established a strong association between HCV and idiopathic pulmonary fibrosis in a different way. They observed 6150 HCV infected patients as test subjects and 2050 hepatitis B virus (HBV) infected patients as controls for the development of IPF over a period of 8.0 ± 5.9 years in the HCV-group and 6.3 ± 5.5 years in the HBV group. In the HCV group, fifteen patients developed IPF compared to none in the HBV group.[28] However, researchers from other countries have failed to replicate these results. Other investigators have also found an association of HCV with a range of fibrotic and non-fibrotic lung diseases.[29]

Other infectious causes of IPF have also been discussed in the literature. The most relevant evidence of IPF was found in patients with previous CMV, EBV, and less commonly Torque-Teno (Transfusion-Transmitted) (TT) virus. Both serological and pathological evidence of herpesvirus family, primarily CMV and EBV infection, was found in patients with IPF. Four studies have found anti-EBV, anti-herpes simplex virus (HSV-1), and anti-cytomegalovirus antibodies, primarily IgG.[11] However, Manika et al. found evidence of IgA in 60% of patients with IPF, compared to 22% of control patients.[31] Tang et al. noted the overwhelming influence of *herpesviridae* in the pathology of IPF, with about 97% of patients with IPF showing CMV, EBV, HHV7, or HHV8 infection(s), compared to 36% of the control patients.[14] Cytomegalovirus, TTV, parainfluenza, rhinovirus, one case of HSV-1, and EBV have caused ARDS in patients with preexisting IPF, COPD, and asthma; acute exacerbations in IPF patients have been fatal.[32–36]

In 2002, when the SARS epidemic began, clinical and radiological evidence of residual pulmonary fibrosis was observed in many of the patients

who survived. A one-month follow-up study on 258 patients suggested that 21.3% had pulmonary diffusion abnormality (D_LCO) < 80% of predicted. The study also showed that the patients who suffered from severe disease process and underwent aggressive treatment are the patients who showed signs of fibrotic changes.[30] A one-year follow-up study done on 97 recovering SARS patients in Hong Kong showed reduced lung function and increased fibrotic changes in 27.8% of SARS survivors compared to the normal population.[37,38] A two-year follow-up study also showed similar findings with a significant reduction in D_LCO, exercise capacity, and lung function tests.[39] In a 15-year follow-up study on patients infected with SARS in 2003, 9% showed evidence of fibrosis following infection. However, that percentage decreased after one year, and it continued to decrease until the 15-year follow-up in 2018, remaining at 3.2%. Ground glass opacities and intralobular and interlobular septum thickening were the most prominent fibrotic features seen in SARS patients at six months and 84 months, respectively.[40] Similar to the SARS-CoV virus, when Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012, a retrospective study done on 36 MERS survivors showed significant radiographic changes in 36%. Lung fibrosis involving one or multiple lobes was seen in 33%, and ground-glass opacities and pleural thickening were found in 5.5%.[41] Another pathologically similar virus, avian influenza A subtype H5N, also caused diffuse alveolar damage (DAD) in patients, but was more acute and hemorrhagic in presentation. Reported cases showed an association between H5N1 infection and development of fibrosis. However, fibrosis associated with H5N1 was less organized than that with SARS.[46]

Pathogenesis of fibrosis in SARS and MERS

During the SARS epidemic in 2003, researchers discovered from autopsies of fatal cases that the major pathological characteristic in SARS patients was DAD, which developed during the early phase of SARS development (7–10 days). Diffuse alveolar damage in SARS is characterized by an inflammatory infiltrate, extensive edema, exudative fluid accumulation, hyaline membrane formation, alveolar collapse, and desquamation.[42] This phase is referred to as the acute phase. After ten days, in the medium phase of SARS development, DAD becomes organized through interstitial and alveolar fibrosis and type II pneumocyte hyperplasia. After 2–3 weeks, the late phase occurs, characterized by extensive fibrous organization and proliferation.[43–50]

The molecular mechanism responsible for fibrosis in SARS is consistent with the pathogenesis of IPF. TGF-β, one of the principal drivers of IPF, was elevated in the early phase of SARS-CoV infection.[51] SARS-CoV-associated damage induced the release of numerous factors, such as extracellular matrix, acute phase reactants, and TGF-β. Thus, high level of TGF-β was observed in damaged lung cells (including pneumocytes, bronchial epithelial cells, and monocytes/macrophages).[52] However, whether it is caused by the virus itself is still unknown.[50]

Furthermore, the virus can induce high levels of serum and tissue TGF-β expression and regulates the signal transduction of the TGF-β pathway through the viral nucleocapsid (N) protein. In alveolar epithelial cells and fibroblasts, overexpression of the N protein leads to TGF-β pathway hyperactivation. This results in excess production of extracellular matrix (ECM) proteins, enhanced secretion of protease inhibitors, such as plasminogen activator inhibitor 1 (PAI-1) and tissue inhibitors of metalloproteinases (TIMP), reduced secretion of proteases, and myofibroblast differentiation and expansion. This ultimately results in increased deposition of the ECM protein and localization of myofibroblasts around active fibrosis sites. Thus SARS-CoV has been shown to cause lung fibrosis through its N protein and resultant activation TGF-β pathway.[53–55]

Another mechanism involves angiotensin-converting enzyme- 2 (ACE2) and angiotensin II (ANG-II) in the development of fibrosis. ACE2 proteins are expressed in various tissues in the human body, including the oral and nasal mucosa, nasopharynx, stomach, small intestine, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain. However, it is most prominently expressed as a surface protein in the alveolar epithelial cells.[56] ACE2 also has a protective role against fibrosis through negative regulation of

local angiotensin level. SARS CoV has a spike protein on its cell surface that works as a receptor for ACE2. Interaction between the S1 subunit of the spike protein and the receptor promotes viral entry and inducing cell to cell fusion.[56] Infection with the SARS CoV has been shown to reduce the ACE2 expression level in the lung epithelial cells.[57] Reduced ACE2 can lead to an increase in the ANG-II levels, which are produced by fibroblasts and activated macrophages and stimulate the secretion of TGF-β from the alveolar epithelial cells, possibly mediated by AGTR1.[56] TGF-β itself can also stimulate ANG-II. Therefore, an "autocrine loop" may be present in the lung tissue.

In addition, ANG-II also activates downstream signaling mediators of the TGF-β pathway, which are SMAD2 and SMAD4 proteins.[57] It can also activate SMAD signaling in a TGF-β-independent but MAPK-dependent manner.[58] In addition, ANG-II can also upregulate connective tissue growth factor (CTGF), promoting ECM deposition and lung fibrosis through the MEK/Erk pathway.[59,60,62] Researchers have found that the ANG-II levels are significantly increased in the lung tissues after administration of the spike protein.[61] Other mechanisms reported to have a role in SARS mediated lung fibrosis are upregulation of monocyte chemoattractant protein-1 (MCP-1) chemokine levels,[63] signal transduction by mitogen-activated protein kinase (MAPK) through viral infection that results in cell differentiation and ECM production, phosphorylation and hyperactivation of p38, which results in actin organization, pulmonary myofibroblast activation, and α-smooth muscle actin (SMA) expression.[64–66] SARS- Co-V infection has been shown to cause EGFR upregulation and enhancement of lung disease in mouse models.[67]

MERS was found more frequently in type-II pneumocytes. Infection with MERS increased fibrotic growth factors, such as SMAD7 and FGF-2, in lung tissue. Both SMAD7 and FGF-2 are responsible for colocalizing caspase-3 expression that eventually results in epithelial cell apoptosis in MERS infected lung tissue.[68]

Clinical evidence of Post-COVID fibrosis

The first autopsy case report of post-COVID fibrosis was published online on July 28, 2020, in a breast cancer survivor woman in her 80s. Despite having no prior history of pulmonary infection, fibrotic lung with bilateral consolidations, septal thickening, traction bronchiectasis and infiltrative and parenchymal changes was found on the 39th day of her hospitalization with COVID-19 infection.[69] Since then, many cases of post-COVID fibrosis have been identified. In a study done on 62 COVID-19 infected patients in Wuhan by Zhou et al., 21 (33.9%) of the patients were found to have fibrotic changes, a finding more likely to be observed in the advanced phase of the disease (8–14 days after onset).[70] Pan et al. also reported fibrotic changes in the CT scans of 17.5% of 63 patients during the acute phase of illness.[71] Postmortem needle core biopsy findings in four patients who died of COVID-19 pneumonia also revealed features of fibroblastic proliferation and deposition of ECM in alveolar spaces along with diffuse alveolar damage.[72] Patients affected by severe COVID-19 infection who later turned negative also had long-term lung dysfunction caused by pulmonary fibrosis. These patients ultimately benefited from lung transplantation.[73]

Pathogenesis of fibrosis in COVID-19

According to recent literature, COVID-19 causes diffuse lung damage through a severe inflammatory response. To mitigate this inflammatory response, regulatory pathways are activated that work toward healing the damaged tissue. Imbalance during this process leads to a fibrotic response of interstitial thickening, ground-glass opacities, irregular interface, coarse reticular pattern, and parenchymal band.[74] Similar to the mechanism of infection in SARS-CoV, SARS-CoV-2 enters the host cells through membrane-anchored ACE2. However, SARS-CoV-2 has a higher binding affinity to ACE2 receptors than that of SARS-CoV.[75] In addition, different proteases, such as transmembrane protease serine 2 (TMPRSS2) and other related proteases, e.g., ADAM17, cause ACE2 cleavage, facilitating the entry of SARS-CoV-2 in the alveolar epithelial cells.[76] Compared to SARS-CoV, soluble ACE2 also has a protective effect against the binding of both SARS-CoV2 and pulmonary fibrosis induced by it. However, critically ill COVID-19 patients, especially those who were elderly, smokers, and diabetic,

had upregulation of ACE2 in their lung tissue. This phenomenon leads to an increased number of viral entry points that ultimately lead to chronic internalization of ACE2 receptors, which increases ANG-II levels with a net increase in proinflammatory and profibrotic cytokines.[77] Prolonged exposure to these cytokines leads to chronic pulmonary fibrosis, along with formation of stiff lung with less compliance. Stiff lung tissues have been found to induce altered cellular response and enhanced deposition of ECM proteins and glycosaminoglycans. All of these factors together lead to progressive pulmonary fibrosis in patients infected with COVID-19.[78]

DISCUSSION OF TREATMENTS FOR **COVID-19** INDUCED FIBROSIS

Pulmonary fibrosis is a progressive and irreversible disease. However, with the right treatment, clinicians have a chance to slow the progression and improve the quality of life. If one can more effectively and efficiently treat the preceding lung disease (e.g., ARDS), this could slow or even stop the initiation of fibrosis in at-risk patients. After the fibrotic changes are underway, appropriate treatment might significantly slow the progression of the fibrosis with the goal of alleviating symptoms and improving morbidity and mortality in patients.

The ARDS Berlin criteria are used by clinicians to categorize and appropriately treat patients who develop ARDS regardless of the cause. However, ARDS secondary to COVID-19 may be different from ARDS secondary to other causes defined by the Berlin Criteria.[79] For example, ARDS due to COVID-19 generally presents 8–12 days after the first symptoms appear;[79] the ARDS Berlin Criteria state that to diagnose a patient with ARDS, the onset must be within one week of a known clinical change. In addition, the main damage caused by SARS-CoV-2 is to the alveolar epithelial cells rather than the capillary endothelial cells as it is in other causes of ARDS.[79] Because ARDS appears to be a major factor in fibrosis development, these differences highlight the need to rethink the treatment given to COVID-19 patients with ARDS.

Although SARS-CoV-2 uses ACE2 receptors to enter cells and cause infection, ACE2 has been reported to have a protective role against lung fibrosis by downregulation of angiotensin 2. Therefore, another potential treatment pathway targets angiotensin 2 to slow the progression of fibrosis. Waseda et al. studied mice with bleomycin-induced pulmonary fibrosis and found that the mice given an angiotensin 2 inhibitor had significantly lower lung fibrosis scores and TGF-beta levels.[80] This study focused on drug-induced pulmonary fibrosis rather than infectious causes of pulmonary fibrosis; therefore, differences in treatment response require more study.

Pirfenidone and nintedanib are two antifibrotic drugs that have proven benefits in patients with IPF. They also demonstrate anti-inflammatory properties that can supposedly be used in the acute phase of COVID-19 pneumonia and ARDS. Pirfenidone can be used theoretically to attenuate LPS induced acute lung injury and resultant fibrosis through NLRP3 inflammasome suppression.[81]

Spironolactone has also shown significant results in the prevention of fibrosis. In animal models, it has antioxidant properties.[82] In several studies, a spironolactone showed alleviation of acute pneumonia through a reduced number of cells, such as lymphocytes, neutrophils, macrophages, and eosinophils, in the alveoli.[82] Jin et al. reported a significant role of spironolactone in the treatment of lung inflammation caused by bleomycin.[83]

In lung fibrosis, not only is there excess production of material secreted into the extracellular matrix, total ECM degradation is also thought to be reduced.[84] Many factors are involved in ECM degradation, especially the plasminogen activator/plasmin system. Plasminogen is the precursor to plasmin, the major factor in fibrin degradation. The activity of plasminogen is regulated by plasminogen activator inhibitor 1 (PAI-1). In fibrotic lungs, PAI-1 expression is increased, hindering normal fibrin degradation. In animal studies, the deletion of the PAI-1 gene led to a reduced susceptibility to fibrosis.[85] This could be a promising treatment in slowing the progression of ongoing fibrosis.

Other novel therapies, such as chitotriosidase 1inhibitor with anti-inflammatory properties, tetrandrine (alkaloid that affects ROS production, calcium channels, and caspase pathways), mesenchymal stem cells (from human purified amniotic fluid that has anti-inflammatory and antifibrotic properties),

hyperbaric oxygen therapy (that reduces the expression of L-1β, IL-6, and TNF-α, reducing ARDS induced fibrosis), lung transplantation, and rehabilitation is also being considered by researchers.[86]

Conclusion

The novel Coronavirus, SARS-CoV-2, and the disease it causes, COVID-19, are proving to be unlike any virus or disease studied thus far. The unprecedented infectivity and transmission has prompted worldwide studies and collaboration to find the best way to treat and prevent the disease. Unfortunately, because this disease is relatively new, there are not much data on long-term complications in patients. However, using the data and knowledge gained from similar viruses, we can predict potential complications and limit future problems. We are learning that there are some aspects of this disease that are not entirely consistent with what we previously thought (e.g., ARDS), and we should suspect that the usual treatment of similar diseases may not be as effective in treating this disease.

To date, most efforts to treat lung fibrosis target proinflammatory mediators, even though there is evidence to show that inflammation is not the primary cause of fibrosis. Future studies should explore the treatment potential of ECM degradation and the reduction of cytokine production and release. There will be many lasting consequences of this pandemic, but if we can mitigate the effects of even one potential consequence, we can improve patients' quality of life and reduce strain on the healthcare system.

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