

**Title:** Similarities and differences in endoplasmic reticulum stress response pathway activation in lung fibrosis in end-stage IPF and COVID ARDS

**Authors:** Samantha Marley, MD, Lauren Sullivan, MD, Poorvi Saini, Xin Sun, PhD, Jamie Verheyden, PhD, Zea Borok, MD, Samvel Gaboyan, Angela Meier, MD PhD, Eugene Golts, MD\*, Kamyar Afshar, MD\*, Laura E. Crotty Alexander, MD\*

**Rationale:** In the aftermath of the SARS-CoV-2 pandemic, numerous studies have evaluated the pathogenesis of lung injury in patients with severe COVID-19. Some patients with acute respiratory distress syndrome (ARDS) due to COVID-19 develop irreversible fibrotic lung disease and ultimately require lung transplantation. The unfolded protein response (UPR) is activated by stress sensed in the endoplasmic reticulum of cells, and dysregulation in this pathway may be implicated in both COVID ARDS-related fibrosis as well as other forms of pulmonary fibrosis. In our study, we used a biobank of low-ischemic time explant specimens from patients undergoing lung transplantation in order to compare UPR pathway proteins in patients with COVID fibrosis, idiopathic pulmonary fibrosis (IPF), and controls.

**Methods:** Nine 1cm<sup>3</sup> cubes were collected from lung explants of patients with pre-transplant diagnoses of COVID-ARDS (n=3), IPF (n=3), and controls (n=6) and were preserved in 10mL of 4% paraformaldehyde. Lung tissue was blocked with 5% goat serum followed by application of primary antibodies: anti-XBP-1, anti-ATF-6, anti-ATF-4, representing the three arms of UPR. Samples were also evaluated for transitional cell markers: keratin (KRT)5, KRT8, and KRT17. IHC stained samples were examined via microscopy to quantify intensity of stain, location, and pattern, indicative of protein expression.

**Results:** XBP-1 was expressed in all three Covid-19 lung samples, one IPF lung, and no controls (Figure 1). KRT5 and KRT17 were present in control, IPF, and COVID-19 lungs, primarily in airway basal cells. Two IPF lungs and all 3 COVID-19 lungs had increased KRT8 expression (Figure 1). The Covid-19 KRT8 was primarily in airway and alveolar epithelial cells. ATF-4 and ATF-6 staining was not present in any samples.

**Conclusion:** XBP-1 and KRT8 have increased expression in both IPF and Covid-19 lung samples. XBP-1 is a known ER stress pathway protein, shown to be involved in the development of IPF, while KRT8 is known to be present in transitional basal cells. Further studies are planned to assess gene expression of ATF-4 and ATF-6. By investigating commonalities between IPF and Covid-19 endoplasmic reticulum stress pathway induction, we can better understand potential drug targets to reduce fibrosis and need for lung transplant.