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# Surfactant Protein-D: The Potential ARDS Biomarker And Its Role In COVID-19

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**ABSTRACT**
As of September 2021, the number of patients diagnosed with COVID-19 worldwide has exceeded 221 million; while the Case Fatality Rate in 215 countries and regions is 2.06%. Acute Respiratory Distress Syndrome (ARDS) is the most common complication of COVID-19 and was reported had a 28-day mortality rate of 74%. Pulmonary injury in ARDS is caused by damage to the alveolar epithelium and pulmonary vascular endothelium caused by both direct virulence of SARS-CoV-2 and an inflammatory reaction that mediates neutrophil activation, leading to diffuse alveolar damage, pulmonary edema and surfactant dysfunction. Under inflammatory conditions, such as ARDS, increased alveolar-capillary permeability may result in alveolar Surfactant Protein-D (SP-D) leakage into the circulation, thus SP-D can be used as a specific biomarker of pulmonary epithelial damage and acute lung injury. SP-D is a specific biomarker of lung epithelial injury and has been shown to correlate with the severity and mortality of ARDS (COVID-19 and non COVID-19). Therefore, the use of SP-D as a prognostic biomarker in COVID-19 cases can be considered.

***Keywords:*** COVID-19; ARDS; surfactant protein-D; biomarker

## INTRODUCTION

As of September 2021, the number of patients diagnosed with COVID-19 worldwide has exceeded221 million; while the Case Fatality Rate in 215 countries and regions is 2.06% (WHO, 2021). Acute Respiratory Distress Syndrome (ARDS) is the most common complication of COVID-19 (60-70% of patients admitted to the ICU), followed by shock (30%), myocardial dysfunction (20-30%), and acute kidney injury (10-30%). (Phua et al., 2020). A study in China even reported that ARDS in COVID-19 had a 28- day mortality rate of 74% (Goh et al., 2020). Surfactant Protein-D (SP-D) is a biomarker of pulmonary epithelial injury, which is produced by type-II alveolar cells and plays an important role in maintaining the integrity of the alveolar-capillary interface (Hartl and Griese, 2006). SP-D is thought to be a more specific marker for lung damage, so it can be used as an early marker of acute lung injury. SP-D has been shown to be a prognostic biomarker to predict poor outcome of H1N1 virus infection and non-COVID- 19 cases (Delgado et al., 2015), but the role of SP-D in COVID-19 cases is not fully understood yet.

## DISCUSSION

### Surfactant Protein-D

The word surfactant comes from the acronym surf(ace) act(ive) a(ge)nt which means superficially active agent (Bracco, 2020). Pulmonary surfactants are complex mixtures of lipids and proteins that form a monomolecular film that coats the alveolar-air interface. Surfactants consist of about 90% lipids (80- 85% of them are phospholipids, 5-10% are neutral lipids) and 10% are proteins. Although proteins make up a small proportion of surfactants, four surfactant- associated proteins, Surfactant Protein (SP)-A, SP-B, SP-C, and SP-D, all have important roles in regulating surfactant function (Glasser and Mallampalli, 2012).

SP-A and SP-D are larger hydrophilic glycoproteins and play an important role in the host defense systems. SP-B and SP-C on the other hand are much smaller polypeptides, are highly hydrophobic, and play an important role in alveolar stability to help reduce surface tension thereby preventing alveolar collapse (Glasser and Mallampalli, 2012). SP-A and SP-D are important components in the function of the host defense system in the lung. These functions include the ability to collect and control pathogen clearance as well as the ability to modify macrophage function. These two proteins are capable of binding to many different pathogens including fungi and yeasts, gram- negative and gram-positive bacteria, mycobacteria, mycoplasmas and viruses (Glasser and Mallampalli, 2012).

SP-D is mainly expressed and secreted by type II alveolar cells and functions to bind and neutralize bacteria, viruses and fungi (Hartl and Griese, 2006). After binding to pathogens, SP-D facilitates pathogen clearance through various mechanisms, one of which acts as an opsonin to increase pathogen clearance through the process of phagocytosis. In addition, SP-D also appears to have an anti-microbial effect on bacteria by increasing the permeability of its membranes. SP-D also initiates phagocytosis indirectly, by stimulating the activity of alveolar macrophages (Glasser and Mallampalli, 2012).

SP-D modulates the host inflammatory response apart from activity against microbial agents. SP-D plays a major role in regulating inflammation by accelerating clearance of apoptotic cells and inhibiting the release of cytokines and other pro-inflammatory products. There are several mechanisms used to clear apoptotic cells. Neutrophil apoptotic cells are cleared through the interaction of SP-D with myeloperoxidase, which is present on the surface of neutrophil cells.

SP-D also interacts with immunoglobulin M to increase phagocytic activity against long-dead apoptotic cells (Glasser and Mallampalli, 2012).

The mechanism by which alveolar SP-D can enters the circulatory system is not clear, but several hypotheses exist: (1) Under inflammatory conditions, such as ARDS, increased alveolar-capillary permeability may result in alveolar SP-D leakage into the circulation; (2) The integrity of the secretory epithelial cells can be damaged in lung inflammation resulting in leakage of SP-D from the epithelial cells into the alveoli and then into the alveolar blood vessels; (3) SP-D has less binding affinity to surfactant lipids than other surfactant proteins, so SP-D can reach the bloodstream easily and produce higher levels of SP-D in serum than SP-A (Hartl and Griese, 2006). SP-D seems to be a good indicator of changes in alveolar epithelial permeability because SP-D is more hydrophilic than other surfactant proteins, so it has a better ability to enter the circulatory system (Ware et al., 2013).

Several studies have investigated the SP-D serum levels as a disease marker for human lung disease. The first comprehensive report showed increased SP-D serum levels in the patients with Interstitial Lung Disease (ILD) compared to healthy controls (Hartl and Griese, 2006). Another recent report showed that SP- D serum levels is useful as a diagnostic tool in severe sepsis patients with ARDS and for evaluating the development of lung injury in critically ill patients on mechanical ventilation. This SP-D serum level was positively correlated with the lung injury score as a parameter to measure the pathological degree of ARDS. A randomized study of critical care patients showed that early alveolar damage can be identified by the presence of SP-D in the blood. A significant increase in SP-D serum levels is a strong independent predictor that a patient has ARDS that will not recover (Jensen et al., 2016). SP-D plays a role in surfactant hemostasis, moreover it also contributes to the regulation of lung tissue inflammation. The presence of an inflammatory process and injury to lung tissue affects the process of synthesis and secretion of SP-D from lung epithelial cells to the systemic circulation. Early identification of lung epithelial damage is one way of detecting the early stages of lung injury before it worsens to ARDS (Veterini, 2020).

**ARDS in COVID-19**

The Berlin definition define ARDS as the onset of a clinical syndrome of symptoms within 7 days of a known cause of illness or new or worsening respiratory symptoms. This combination of clinical symptoms consists of acute hypoxaemia ($PaO_2/FiO_2$ ratio<300 mmHg), in a ventilatory patient with a positive end-expiratory pressure of at least 5 $cmH_2O$, and bilateral opacity that is not caused by heart failure or volume overload. The Berlin criteria uses the $PaO_2/FiO_2$ ratio to differentiate mild ARDS ($200<PaO_2/FiO_2\leq300$ mmHg), moderate ARDS ($100<PaO_2/FiO_2\leq200$ mmHg), and severe ARDS ($PaO_2/FiO_2<100$ mmHg) (Papazian et al., 2019). WHO categorizes ARDS in COVID-19 as a critical illness degrees and uses the same definition and classification of ARDS degrees as the Berlin criteria (WHO, 2020) ARDS is the leading cause of death in critical care units worldwide, with a mortality rate of around 40% even with recent treatment advances. Overall, the mortality rate for ARDS in the ICU was 35.3%, which was higher as the severity of ARDS increased (Spadaro et al., 2019). ARDS is the most common complication of COVID-19 (60-70% of patients admitted to the ICU) and has a 28-day mortality rate of 74% (Phua et al.,2020; Goh et al., 2020). The most common causes of ARDS are pneumonia and sepsis, but other unknown factors also play a role in the pathogenesis.

Among them genetic factors, virulence factors and environmental factors (such as exposure to mechanical ventilation resulting in lung injury) can contribute to the development of the disease into ARDS (Spadaro et al., 2019).

The pathogenesis of the coronavirus is still not fully understood. Cytokine storm and viral evasion mechanisms of cellular immune responses are thought to play an important role in determining the disease severity. Neutrophilia was found in the blood serum and lungs of patients infected with SARS-CoV-2. In MERS, the severity of lung injury correlates with extensive neutrophil and macrophage infiltration in lung tissue and blood serum levels. Neutrophils are the main source of chemokines and cytokines. Cytokine storms that lead to ARDS are the leading cause of death in patients with SARS and MERS. In one study, COVID-19 patients with ARDS had significantly higher neutrophil counts than those without ARDS, perhaps neutrophil activation is not only aimed at regulating the immune response to the virus, but also contributes to the occurrence of cytokine storms. This finding may partly explain the positive association between high fever and ARDS found in the early stages of COVID-19 (Wu et al., 2020).

ARDS COVID-19 is a disease characterized by severe hypoxemia which is often accompanied by near- normal lung compliance. Gattinoni hypothesized that the different patterns of ARDS in COVID-19 depend on the interaction between three factors: (1) severity of infection, host response, physiological reserve and comorbidities; (2) the patient's ventilatory response to hypoxaemia; (3) time between disease onset and hospitalization (Gattinoni et al., 2020).

Pulmonary injury in ARDS is caused by damage to the alveolar epithelium and pulmonary vascular endothelium caused by both direct virulence of SARS- CoV-2 and an inflammatory reaction that mediates neutrophil activation, leading to diffuse alveolar damage, pulmonary edema and surfactant dysfunction. Damage to the pulmonary alveolo- capillary membrane, one of which is caused by impaired regulation of the host response to infection, resulting in clinical disorders in the form of hypoxemia, inflammation and non-cardiogenic pulmonary edema (Veterini, 2020).

The Role of Surfactant Protein-D in COVID-19 Several studies of SP-D in patients with COVID-19 and other viral infections have been conducted. A 2015 study by Delgado examined SP-D in 37 patients infected with the H1N1 virus. It shows that the overall mean value of SP-D is 434.5 ng/mL, in the non- survived group it is 630 ng/mL, in the survived group it is 172 ng/mL while in the control group it is 49.5 ng/mL. This study showed that SP-D serum levels were significantly higher in patients who died (630 ng/mL) when compared to survivors (172 ng/mL, $p<0.02$) and healthy controls (49.5 ng/mL, $p<0.0001$). This study concluded that higher SP-D serum levels were associated with a higher risk of death in patients with pneumonia due to H1N1 virus infection (Delgado et al., 2015).

The study by Kerget on 88 COVID-19 patients that was examined for SP-D serum levels on day 0 and day 5 since hospital admission. This study examined SP-D serum levels in COVID-19 patients with ARDS and without ARDS compared to the control group. The mean SP-D serum levels in the control group was 21.1ng/mL, while the mean SP-D on day 0 of the ARDS group was 83.3 ng/mL and 46.5 ng/mL for non ARDS group. Measurement of SP-D serum levels was carried out again on day 5, where the ARDS group got 46.4 ng/mL and the non ARDS group got 22.4 ng/mL.

The results of this study showed that in COVID-19, SP-D serum levels were higher in patients with ARDS than in those without ARDS (p=0.001). Likewise, the SP-D measurements in the non-survived versus survived group (96.7 ± 37.2 ng/ml vs 56.9 ± 43.5 ng/ml, p=0.03) (Kerget et al., 2020).

Study by Saito examined SP-D serum levels in 46 COVID-19 patients. This study divided the sample into groups of severe and mild disease degrees and then observed SP-D serum levels on days 3, 6 and 8. The results showed that SP-D serum levels in mild compared to severe cases had a significant difference (61.9 ± 50.7 vs. 237 ± 210, p < 0.001). In addition, this study also stated that in the severe cases group, the increase in SP-D serum levels on day 8 when compared to day 3 was 8.5 times higher where the increase in SP- D serum levels was also accompanied by worsening clinical condition (Saito et al., 2020).

The SP-D serum levels in COVID-19 is generally lower when compared to H1N1 cases. This may be due to the pathophysiological process of COVID-19 itself, where it is known that SARS CoV-2 enters the respiratory tract and binds to the ACE-2 receptor on type II alveolar cells. Type II alveolar cells function is to produce surfactant, so damage to these cells early in the course of the disease can cause a decrease the amount of pulmonary surfactant. This causes the number of SP-Ds that leak into the systemic circulation is not much because the number of SP-Ds in the lungs from the beginning has decreased. This is supported by a study conducted by Arroyo, who found that COVID-19 patients exhibited decreased pulmonary SP-D serum levels (median = 68.9 ng/mL) compared to SP- D serum levels reported in the literature for healthy subjects, which ranged from 900-1300 ng/mL (Arroyo et al., 2021).

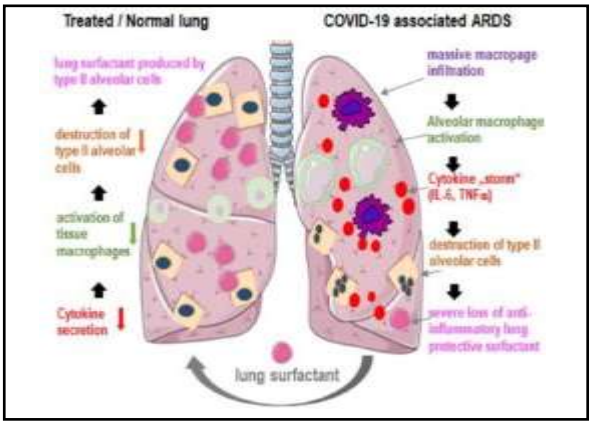


**FIGURE 1:** Hypothesis of Surfactant Therapy Effect Mechanism (Ursula Mirastschijski, 2020).

In addition to the role of SP-D as a biomarker that capable of describing the severity of ARDS in COVID- 19, there are also opinions that support the possibility that surfactant therapy may provide benefits in COVID-19 cases. Although pulmonary surfactant therapy is standard, safe and effective therapy for neonates with ARDS, treatment with recombinant SP- C-based surfactant has not shown an increase in survival in a randomized controlled trial in adults (Meng et al., 2019). The use of natural surfactants seems to be more advantageous than synthetic surfactants in increasing blood oxygenation significantly and shortening ventilatory time in infant patients. Meconium aspiration syndrome resembles COVID-19 pneumonia in which there is decreased surfactant production due to destruction of type II alveolar cells. Early administration of natural surfactants reduces the need for ECMO therapy and ventilatory time in infant patients.

This suggests that initial administration of natural surfactants should also improve lung function in adult patients with severe ARDS. Thus, surfactant therapy in ARDS patients due to COVID-19 may be of benefit, especially when applied early in the disease course (Ursula Mirastschijski, 2020).

ARDS due to COVID-19 is characterized by massive infiltration of macrophages, activation of alveolar macrophages and potentiation of cytokine production in the lungs causing a cytokine storm, leading to destruction of surfactant-producing type II alveolar cells, whereas decreased surfactant also causes loss of anti-inflammatory function and anti-fibrosis. Exogenous surfactants are expected to reduce inflammation and thereby aid the regeneration of the lung. By covering the outer surface of the alveoli, pulmonary surfactants act directly on the inflammatory cells and thereby also reduce cytokine production and tissue destruction. Exogenous surfactants are also expected to restore lung protective function and prevent lung collapse (Figure 1). The end result is expected to reduce the duration of ventilator therapy, facilitate breathing and oxygenation, thereby contributing to patient recovery (Ursula Mirastschijski, 2020).

## CONCLUSION

SP-D is a specific biomarker of lung epithelial injury and has been shown to correlate with the severity and mortality of ARDS (COVID-19 and non COVID-19). Therefore, the use of SP-D as a prognostic biomarker in COVID-19 cases can be considered.

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