CAN PULMONARY SURFACTANT PROTEINS BE RELIABLE INDICATORS OF COVID-19-ASSOCIATED PULMONARY INJURY?

Received: May 2, 2022
Accepted: June 29, 2022

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Abstract:
The COVID-19 pandemic is still raging all over the world. New variants of the coronavirus emerge and infect recovered from previous infections, vaccinated, and unvaccinated subjects. One aspect remains unchanged that is the lungs are the main targets of the pandemic coronavirus. This challenging situation requires the search for reliable predictive markers of severe and complicated course of the disease. Serum surfactant proteins are known to correlate with pulmonary injury severity in numerous diseases. Measurement of such protein levels may help timely predict the risk. Surfactant proteins can also be helpful diagnostic purposes in COVID-19.

Keywords: SARS-CoV-2, Inflammation, Surfactant proteins, Alveolar type II cells, COVID-19


INTRODUCTION
The coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a dramatic challenge for the global healthcare system [1]. The lungs are considered one of the main target organs affected by COVID-19 owing to its rich vascularized area [2]. The pulmonary system requires specific monitoring during the observation of COVID-19 progression. Increasing cases of SARS-CoV-2 draw the public health system's attention to look for specific markers identifying pulmonary system involvement. From a pathophysiological point of view, the changes in the surfactant system undoubtedly affect the whole respiratory process during the disease progression. Our review aims to shed light on the roles of surfactant proteins as valuable biomarkers in COVID-19 patients.

SEARCH STRATEGIES
We conducted the searches on MEDLINE/PubMed and Scopus for the literature data on COVID-19 in infected patients according to the recommendations [3]. The literature search was confined to peer-reviewed
manuscripts and was restricted to English-language articles.

We used the following keywords: “surfactant proteins”, “pulmonary surfactant”, “surfactant protein A”, “surfactant protein B”, “surfactant protein C”, and “surfactant protein D” in various combinations with “COVID-19” or “SARS-CoV-2”.

SURFACTANT STRUCTURE
In 1957, Clements identified and shed the light on the pulmonary surfactant as a lipid-protein complex produced by type II alveolocytes (AE2C) [4]. The main role of surfactants is to create a film on the respiratory surface and to provide stability during the phases of respiration [4].

ATII cells provide homeostasis in the alveolar-capillary barrier and preserve the alveoli stability by regulating the sodium channels and making the alveoli free of fluid [5]. The surfactant in its structure has four corresponding surfactant proteins, namely, SP-A, SP-B, SP-C, and SP-D [6].

SP-A and SP-D are considered to be hydrophilic, and the other two are hydrophobic [6]. The main roles of SP-A and SP-D are mainly provided due to binding to viruses and eliminating them via phagocytosis [6]. SP-A and SP-D are collectins, cooperating with pathogens through lectin domains [7]. Additionally, SP-A and SP-D owe immunological properties in the lungs by enhancing or inhibiting the production of inflammatory molecules [8].

It is worth noting the diversity of SP-D effects. SP-D may owe a dual role in lung protection: SP-D can either suppress or exacerbate pneumonia. This depends on whether SP-D binds to alveolar macrophages via a carbohydrate recognition domain (CRD)-dependent or CRD-independent method [9].

Notably, the hydrophobic SP-B and SP-C play a significant role in the biophysical constancy of surfactant films. They reduce the alveolar surface tension required to avert collapse [10]. Following Laplace’s law, the constancy in the alveoli of different sizes is maintained due to reduced tension of the surface with the help of surfactant [11]. Thus, surfactant deficiency results in increased surface tension and the occurrence of atelectasis with further impairment of gas exchange [12].

THE ROLE OF SURFACTANT IN DIFFERENT DISORDERS
It has been noted that surfactant proteins are present almost exclusively in the lungs, and the increase in their number in the blood can be postulated by increased permeability of pulmonary vessels or damage of pulmonary structures. This hypothesis was proved in the authors’ research where elevated serum SP-D levels were observed in patients with systemic sclerosis, interstitial lung disease, as well as in patients with idiopathic pulmonary fibrosis [13]. In patients with idiopathic pulmonary fibrosis and sarcoidosis, the determination of serum SP-D levels predicted the degree of parenchymal lesions and the survival of these patients [14].

Analysis of SP-D showed high rates of severe acute respiratory syndrome (SARS) which terminated in a severe outbreak in 2003 [15]. In case of SARS-type pneumonia ((95% CI) 453 (379–963) ng/ml compared with controls 218 (160–362) ng/ml, p < 0.05) [21]. Furthermore, a significant correlation of SP-D with anti-SARS-CoV N protein IgG (r^2 = 0.5995, p = 0.02) suggests the dual role of SP-D in innate and adaptive immunities [15].

The importance of the evaluation of surfactant proteins might show its relevance, especially in patients with comorbidities. Increasing evidence suggests that diabetes mellitus, which is one of the most common comorbidities in COVID-19, has a tight link with SARS-CoV-2. Due to that fact, such patients have an increased risk of severe COVID-19 manifestations and must be precisely followed up by medical specialists [16].

Scientists have studied the relationship between the level of SP-D in the serum of patients with type 2 diabetes with obesity and respiratory disorders. It was found that there is an inverse relationship between the volume of forced exhalation in the first second (FEV1) and the level of SP-D in the serum (p = 0.029) [17]. These results suggest the validity of monitoring serum SP-D in patients with type 2 diabetes with obesity at the first stage of the screening test when they are infected with COVID-19.

THE ROLE OF SURFACTANT IN COVID-19
SARS-CoV-2 entry depends on characteristic affinity to the Angiotensin-converting enzyme 2 receptor (ACE2) which is highly expressed on epithelial cells lining the alveolar and bronchi surface [18].
SP-B protein is produced in the lamellar bodies of type II epithelial cells [9]. However, the same cells express ACE2 receptor for SARS-CoV-2 virus entry [20], suggesting the possible imbalance of surfactant secretion during COVID-19 progression.

Regarding COVID-19 clinical severity, Alay and colleagues observed that SP-D levels were significantly higher in COVID-19 patients with mild-moderate pneumonia and in patients with the critical form than in those with the asymptomatic form of COVID-19 (p < 0.001 for all groups) [21]. Furthermore, a positive significant correlation was found between the clinical severity of COVID-19 and serum SP-D (r = 0.885 p < 0.001) [21].

ROC analysis showed a cut-off value of SP-D of 37.7 ng/ml (AUC = 0.763, p < 0.001, 95% confidence interval [CI] = 0.667–0.860), suggesting that SP-D might serve as a good predictive marker of COVID-19 severity [21].

Additionally, the clinical features of community-acquired pneumonia (CAP) seemed to be quite similar to those presented in COVID-19 patients [22]. However, severe COVID-19 patients had higher serum levels of SP-D than patients with CAP (p < 0.001) [21]. These results show potential in making the differentiation between diagnoses.

**PREDICTION OF CONSEQUENCES WITH THE HELP OF SP-D RATES**

The serum SP-D levels seem to be able to predict COVID-19 consequences. Interesting results were found on univariate analysis: on the 1st day of hospitalization, the SP-D levels showed a positive association with further development of ARDS (OR: 1.006 [1.003–1.009]; p < 0.001) [23]. These associations were confirmed on a multivariate analysis after adjusting for the contributing factors [23].

Additionally, in COVID-19 hospitalized patients, there was an increase in serum SP-D level on the 5th day (296.3 [162–541.8] ng/ml; p < 0.001) and on the 14th day (242.8 [105.1–560.8] ng/ml; p = 0.007) in comparison with the values on the 1st day (144.9 [55–381.5] ng/ml) [23].

**SP-D IN DIFFERENTIATING DIFFERENT TYPES OF PNEUMONIA**

Another research was conducted to evaluate the role of serum SP-D in differentiating COVID-19 pneumonia from pneumonia-like diseases that can show clinical similarity [24]. Interestingly, the patients infected with COVID-19 pneumonia had significantly lower rates of SP-D levels than the patients with COVID-19 pneumonia-like disease (24.7 [8.6–51.0] ng/ml vs. 141 [63.7–243.5] ng/ml, p < 0.0001) [24].

Additionally, they identified shortened time between clinical symptoms presentation and estimation of SP-D in patients with COVID-19 pneumonia compared with those with non-COVID-19 pneumonia (7 [4–9] days vs. 10 [5–16] days, p = 0.036) [24].

Blood test analysis showed lower white blood cell counts in patients with COVID-19 pneumonia than in patients with non-COVID-19 pneumonia-like diseases (4850 [3675–6400]/μl vs. 7500 [6050–10,100]/μl, p < 0.0001) [24]. The ROC analysis showed that the area under curves (95% CI) of serum SP-D levels for differentiation between COVID-19 pneumonia from non-COVID-19 pneumonia-like diseases was 0.874 (0.812–0.936). The cut-off level for serum SP-D was 63.7 ng/ml with sensitivity and specificity of 75.4% and 85.2%, respectively [24].

**ASSOCIATION BETWEEN PROINFLAMMATORY CYTOKINES AND SP-D**

Activation of inflammatory pathways with damage to capillaries leads to the development of acute respiratory distress syndrome (ARDS) and impairment of multiple organs in COVID-19 patients with complicated clinical course [7]. It has been known that COVID-19 deterioration from mild to severe clinical forms coexist with the remarkable elevation of serum IL-6 levels due to an imbalance between CD4+ and CD8+ cells [25].

Accordingly, another study was performed to identify the link between serum interleukin 6 (IL-6) and SP-D levels and the clinical course and prognosis of COVID-19 [26]. On admission, both biomarkers were significantly higher in non-survivors than in those who survived (IL-6: p = 0.001, SP-D: p = 0.03) [26]. Additionally, patients who developed macrophage activation syndrome (MAS) showed significantly elevated serum levels of IL-6 and SP-D on admission and on day 5 of follow-up compared with patients without MAS (IL-6: p = 0.001 for both; SP-D: p = 0.02, p = 0.04) [26].
Overall, impairment of the surfactant system was justified in pulmonary histopathological findings. There were 18 COVID-19 individuals who showed diffuse damage of the alveolar-capillary barrier with depletion of surfactant protein expression. Also, there was an increase in plasminogen activator inhibitor-1 in plasma, suggesting the violation of clot fibrinolysis [27].

**MONITORING SURFACANT PROTEIN LEVELS AFTER COVID-19 HOSPITAL DISCHARGE**

Epithelial markers (SP-A, SP-D) can be elevated for a prolonged period after hospital discharge, indicating severe impairment of the alveolar-capillary barrier and violation of respiratory function [28].

Sibila et al. determined the levels of SP-A, SP-D, and sICAM-1 markers 6 months after hospital discharge in 215 patients treated previously for COVID-19. SP-A (p = 0.004), SP-D (p = 0.002), and sICAM-1 (p = 0.025) were significantly higher in individuals with reduced DLCO than in patients with normal DLCO values. Circulating SP-A levels were linked with the occurrence of ARDS and pulmonary embolisms during hospitalization [28].

**SP-D LEVELS FOR STRATIFICATION OF COVID-19 PATIENTS**

Accumulating evidence suggests that SP-D is a particularly valuable marker for the stratification of COVID-19 patients according to their severity [29]. In the acute period, higher SP-D levels were seen in severe patients compared with mild ones (449.7 ± 125.8 vs. 245.9 ± 90.0 ng/mL, p < 0.001). Compared with the acute phase, in the recovery period, SP-D decreased significantly in the mild group (194.5 ± 66.1 vs. 120.0 ± 50.0 ng/mL, p < 0.001) as well as in severe patients (449.7 ± 125.8 vs. 119.8 ± 45.2 ng/mL, p < 0.001) [29].

Elevated SP-D levels of severe patients were in line with elevated CT imaging scores which could also be used for the stratification of patients [29]. SP-D had a positive correlation with CRP (r = 0.658, p < 0.001) and IL-6 (r = 0.471, p = 0.002), whereas it showed a negative correlation with LYM (r = −0.320, p = 0.047) [29]. Reliability of SP-D as a severity marker was shown in ROS analysis. The AUC of SP-D was 0.922 (95%CI 0.833–1.000, p < 0.001) with a cut-off of 309.7 (sensitivity 88.9%, specificity 86.7%) [29]. Additionally, in bronchoalveolar lavage fluid (BALF), decreased levels of SP-D in severe COVID-19 patients were detected [30].

To identify the correlation between SP-D with ARDS severity in patients with COVID-19, another study was conducted. The correlation analysis showed a positive correlation between SP-D and ARDS severity upon admission (r = 0.236; p = 0.04) [31]. The valuable role of surfactant to provide its normal respiratory function could also be explained by the positive effect of vitamin D in COVID-19 management. It has been shown that supplementation of vitamin D promotes the synthesis of surfactant [32].

**THERAPEUTICALLY POTENTIAL ROLE**

Consistent with its role as a biomarker, the prospective role of a recombinant fragment of human SP-D (rfhSP-D) to deal with SARS-CoV-2 infection was shown in another study. The scientists found that rfhSP-D suppressed the interaction between S1 protein (surface glycoprotein perceived by innate immunity) and HEK293T cells with increased expression of human angiotensin-converting enzyme 2 (hACE2) [33]. Therapeutical strategies were shown from another point of view: rfhSP-D acted as an inhibitor for SARS-CoV-2 entry [33].

**CONCLUSIONS**

In this overview, we highlighted the roles of surfactant proteins as markers of disease progression and follow-up after hospital discharge. Monitoring the values of surfactant proteins in COVID-19 may enrich information about the current respiratory status of infected patients and predict deterioration. Researchers may consider the relevance of further studies on surfactant proteins to fully clarify their roles in COVID-19.

**References**


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Table 1. Peculiarities of serum surfactant protein levels in COVID-19 patients

<table>
<thead>
<tr>
<th>Surfactant protein</th>
<th>Country</th>
<th>Peculiarities</th>
<th>References</th>
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<tbody>
<tr>
<td>SP-D</td>
<td>Turkey</td>
<td>- Serum SP-D levels in critical COVID-19 patients were significantly higher than those in CAP patients (P&lt;0.001). - Positive correlation between severity of COVID-19 and SP-D levels (r=0.885 P&lt;0.001) - ROC curve analysis for SP-D: cut-off value of 37.7 ng/ml (AUC =0.763, P&lt;0.001, 95% CI 0.667–0.860).</td>
<td>[21]</td>
</tr>
<tr>
<td>SP-D</td>
<td>Belgium</td>
<td>- Higher SP-D levels in critically-ill patients on day 1 (304.3 [134.4–888.8] ng/ml) than in the outpatient group (73.2 [50.2–106.8] ng/ml; P&lt;0.001); hospitalized group (144.9 [55–381.5] ng/ml; P=0.002); convalescent group (86.7 [46–163.4] ng/ml; P&lt;0.001). - Higher SP-D levels in hospitalized patients on day 5 (296.3 [162–541.8] ng/ml; P&lt;0.001) and day 14 (242.8 [105.1–560.8] ng/ml; P=0.007) than the levels on day 1 (144.9 [55–381.5] ng/ml).</td>
<td>[23]</td>
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<td>SP-D</td>
<td>Japan</td>
<td>- Significantly lower SP-D levels in patients with COVID-19 pneumonia than in patients with COVID-19 pneumonia-like disease 24.7 [8.6–51.0] ng/ml vs. 141 [63.7–243.5] ng/ml, P&lt;0.0001). - ROC curve analysis for SP-D levels for differentiation between COVID-19 pneumonia from COVID-19 pneumonia-like diseases AUC 0.874 (95% CI 0.812–0.936).</td>
<td>[24]</td>
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<tr>
<td>SP-A</td>
<td>Spain</td>
<td>Significant correlation between DLCO values and SP-A, rho= −0.32, P&lt;0.001.</td>
<td>[28]</td>
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<tr>
<td>SP-D</td>
<td>Spain</td>
<td>Significant correlation between DLCO values and SP-D, rho=−0.25, P&lt;0.001.</td>
<td>[28]</td>
</tr>
<tr>
<td>SP-D</td>
<td>China</td>
<td>- Higher levels of t SP-D in patients with severe COVID-19 clinical course than in the mild group (mean value ± standard deviation (SD), 449.7 ± 125.8 vs 245.9 ± 90.0 ng/mL, P&lt;0.001). - Sharp reduction in serum SP-D values in the recovery period compared with the acute phase (mean value ± SD, 129.5 ± 51.7 vs 292.9 ± 130.7 ng/ml, P&lt;0.001).</td>
<td>[29]</td>
</tr>
<tr>
<td>SP-D</td>
<td>Indonesia</td>
<td>Significant positive correlation between SP-D serum values and ARDS severity upon hospital admission (rho=0.26, P=0.04).</td>
<td>[31]</td>
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СУРФАКТАНТ АКУЫЗДАРЫ COVID-19 БАЙЛАНЫСЫ ТОКО ПАЙДА ЛАЙ БУЛУНУН СЕНІМДІ ИНДИКАТОРЫ БОЛА АЛАДЫ МА?

Түйіндеме


Дейінгі ушін: Қыңк жаңа дәреже ауыр және құрделе аясының семінді бөлігі болмаған штамлар. Вакцинацияланған, вакцинацияланған және егілгендерге жұқаты коронавирустары жаңа штамдары пайда болуына. Бұл аспект еңбек: оқпесіз сурфактанттық ауруларда қызмет жасайды. COVID-19 кезінде сурфактант ақуыздары диагностикалық максаттарда пайдалы болуы мүмкін.

МОГУТ ЛІ БЕЛКІ СУРФАКТАНТА В ЛЕГКИХ БЫТЬ НАДЕЖНЫМИ ИНДИКАТОРАМИ ПОРАЖЕНИЯ ЛЕГКИХ, СВЯЗАННОГО С COVID-19?

Резюме
Пандемия COVID-19 продолжает бушевать по всему миру. Появляются новые штаммы коронавируса, которые заражают выздоровевших от предыдущих штаммов, вакцинированных и непривитых. Один аспект остается неизменным: легкие являются основными мишенями пандемического коронавируса. Сложная ситуация требует поиска надежных прогностических маркеров тяжелого и осложненного течения заболевания. Измерение таких уровней белка может помочь своевременно предсказать риск. Белки сурфактантта также могут быть полезны в диагностических целях при COVID-19.

Ключевые слова: SARS-CoV-2, воспаление, белки сурфактантта, альвеолярные клетки II типа, COVID-19