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Immunohistochemical findings in the lungs of COVID-19 subjects: evidence of surfactant dysregulation

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Abstract. – OBJECTIVE: Acute respiratory distress syndrome (ARDS) is characterized by quantitative and qualitative changes in surfactant composition, leading to surfactant dysregulation with alveolar collapse and acute respiratory hypoxic failure. Recently, surfactant has been hypothesized to play a relevant role in COVID-19, representing a strong defender against SARS-CoV-2 infection. The aim of our work was the study of immunohistochemical surfactant expression in the lungs of patients died following SARS-CoV-2 ARDS, in order to shed light on a possible therapeutic surfactant administration.

PATIENTS AND METHODS: We investigated four patients who died due to ARDS following SARS-COV-2 infection and four patients submitted to lung biopsy, in the absence of SARS-CoV-2 infection. In all 8 cases, lung specimens were immunostained with anti-surfactant protein A (SP-A) and B (SP-B).

RESULTS: In control subjects, reactivity for SP-B was restricted to type II alveolar cells. Immunostaining for SP-A was observed on the surface of alveolar spaces. In the COVID-19 positive lungs, immunoreactivity for SP-B was similar to that observed in control lungs; SP-A was strongly expressed along the alveolar wall. Moreover, dense aggregates of SP-A positive material were observed in the alveolar spaces.

CONCLUSIONS: Our immunohistochemical data show the dysregulation of surfactant production in COVID-19 patients, particularly regarding SP-A expression. The increased presence of SP-A in condensed masses inside alveolar spaces could invalidate the therapeutic efficacy of the treatment with exogenous surfactant.

Key Words:
COVID-19, Surfactant, Hyaline membranes, Surfactant dysregulation, ARDS.

Introduction

According with the Berlin Definition, Acute Respiratory Distress Syndrome (ARDS) is the acute onset of hypoxemia, which may be subdivided into 3 categories (mild, moderate and severe) based on the degree of hypoxemia[1]. ARDS is characterized by quantitative and qualitative changes in surfactant composition, leading to surfactant dysregulation with alveolar collapse and acute respiratory hypoxic failure[2]. Early administration of surfactant, to preterm infants affected by the neonatal respiratory distress syndrome (NRDS), has been shown to be beneficial, decreasing the risk of perinatal mortality[3]. Moreover, exogenous surfactant therapy has been

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reported to be beneficial even in at term infants affected by pneumonia or meconium aspiration and in children with ARDS[4]. On the contrary, in adult subjects with ARDS, the administration of surfactant therapy remains a challenge, with no evidence of significant treatment benefit[5,6]. Some studies[6] evidenced that exogen surfactant treatment in patients with (ARDS) had limited success, possibly due to the complexity of that syndrome. Adult patients with ARDS have failed to exhibit positive effects on morbidity and mortality[7,8].

Endogenous surfactant consists of ~80% phospholipid, 7-10% neutral lipids and ~10% surfactant-associated proteins[9].

The protein fraction of surfactant comprises 4 molecules, defined as surfactant protein- B (SP-B), SP-C, SP-A and SP-D. The first two are hydrophobic and reduce surface tension improving indexes of oxygenation, while SP-A and SP-D are hydrophilic and play relevant immunomodulatory roles[10,11].

In physiology, pulmonary surfactant lines the alveolar surface and reduces the surface tension[12]. Because of its strategic position, pulmonary surfactant also plays an essential role in the host defense mechanisms when airways meet pathogens[13].

ARDS is characterized by surfactant dysregulation with loss of function. This alteration is likely multifactorial, being related to: i) injury to type II alveolar epithelial cells, the main producers of surfactant proteins; ii) inhibition caused by proteins contained in the alveolar edema fluid; iii) conversion of surfactant into an inactive form; iiii) changes in surfactant components due to inflammation[5].

Recently, surfactant has been hypothesized to play a relevant role in COVID-19, representing a strong defender against SARS-CoV-2 infection. According with this hypothesis, SP-A and SP-D might bind to SARS-CoV-2 to facilitate pathogen removal and neutralization, could enhance phagocytosis and agglutination of the virus, preventing infection of alveolar epithelial type II (AE2) cells[14].

Patients with COVID-19, despite meeting the Berlin definition of ARDS, present an atypical form of the syndrome[15]. This study is aimed at analyzing by immunohistochemistry surfactant expression in the lung of patients died following SARS-CoV-2 ARDS, in order to shed light on a possible surfactant dysregulation in COVID-19 patients at tissue level.

Patients and Methods

A retrospective analysis on lung specimens from 4 patients died due to ARDS following SARS-COV-2 infection was performed. As control group, 4 patients submitted to lung biopsy, with absence of SARS-CoV-2 infection and with preserved architecture, were utilized.

Tissue samples were routinely processed for histological observation and stained with hematoxylin and eosin.

For Immunohistochemical analysis, 3 μm thick sections were obtained from each paraffin block. All reagents, except the anti-Surfactant A and the anti-Surfactant B-(Precursor) antibodies, were purchased from Ventana Medical Systems Inc. 1910 E. Innovation Park Drive Tucson, Arizona 85755 USA.

Sections to be incubated with the primary antibodies were first automatically dewaxed and rehydrated with EZ Prep 1X (Ref. 950-102) and pre-treated at 95°C for 52 minutes with heat-induced epitope retrieval in Ultra CC1 (Ref. 950-224), following Dealer's instructions.

Slides were then incubated for 20 minutes at room temperature with anti-Surfactant Protein A/PSAP mouse monoclonal antibody (clone 6F10 (Ref. ab51891 Abcam - Discovery Drive, Cambridge Biomedical Campus, Cambridge CB2 0AX, UK) at 1:6000 dilution and for 32 minutes at room temperature with anti-Surfactant B (Precursor) mouse monoclonal antibody (clone SPB01 (Ref. MS-704-P1 – NeoMarkers Inc. -47790 Westinghouse Dr, Fremont, CA 94539, United States) at 1:50 dilution.

Counterstaining was performed incubating sections for 8 minutes with Haematoxylin II (Ref. 790-2208) and for 4 minutes with Bluing Reagent (Ref. 760-2037).

All immunostaining procedures were performed using the UltraView Universal DAB Detection Kit (Ref. 760-5000) on the BenchMark Ultra (Ventana Medical Systems Inc. 1910 E. Innovation Park Drive Tucson, Arizona 85755 USA) instrument, according to the manufacturer's instructions.

Results

Microscopic examination of COVID-19 lungs revealed reduction of alveolar spaces or atelectasis zones with presence of hyaline membranes.

Immunostaining for SP-B, in control normal lungs was characterized by a reactivity in the type II alveolar epithelial cells. The reactivity was cytoplasmatic or membranous. Focal reactivity for SP-B was also observed in scattered bronchial cells (Figure 1).

In all four cases of COVID-19 ARDS, we observed marked proliferation of alveolar type II epithelial cells. All these cells showed immunostaining for SP-B in the cytoplasm and along the cell membrane (Figure 2).

Immunostaining for SP-A in normal control lungs was characterized by a reactivity in alveolar type II epithelial cells. Moreover, a thin deposition of SP-A was observed along the alveolar walls (Figure 3).

In all four cases of COVID-19 ARDS, SP-A was found in the alveolar type II epithelial cells and strongly expressed along the alveolar walls. Moreover, with condensed SP-A positive masses were observed within the alveolar spaces (Figure 4).

Discussion

SARS-CoV-2 enters and replicates in the alveolar type I and type II cells. The consequences are hyperplasia of type II pneumocytes[16] and dysregulation of pulmonary surfactants[16].

Conflicting results have been published regarding a possible surfactant dysregulation in the lung of COVID-19 patients.

Some authors observed surfactant depletion correlated to SARS-CoV-2 induced lysis of type II pneumocytes and to hyaline membranes formation[17].

Heching and coworkers suggested that SARS-Co-2 damage type II alveolar cells, inhibiting production of surfactant[18]. According with Bollag and coworkers, COVID-19-infected patients could benefit surfactant administration with reduction of pulmonary injury[19].

On the other hand, it has been claimed that there is no direct evidence that surfactant is dysfunctional in the lungs of COVID-19 patients. Indirect evidence suggests that surfactant dysfunction might represent a significant contributing factor to the pulmonary injury in COVID-19 patients[6].

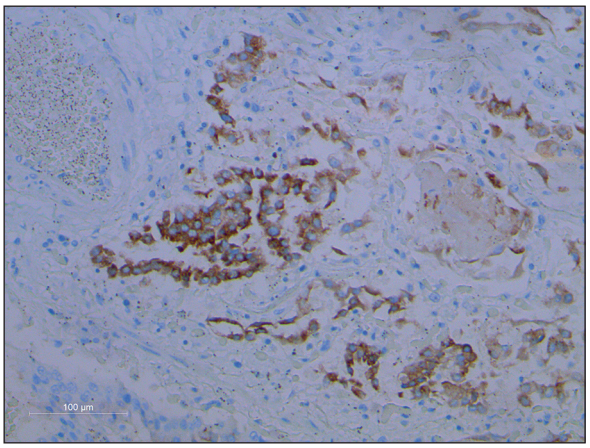

Figure 2. Surfactant Protein B in COVID-19 lungs (20×): marked positivity for SP-B in hyperplastic alveolar type II epithelial cells.

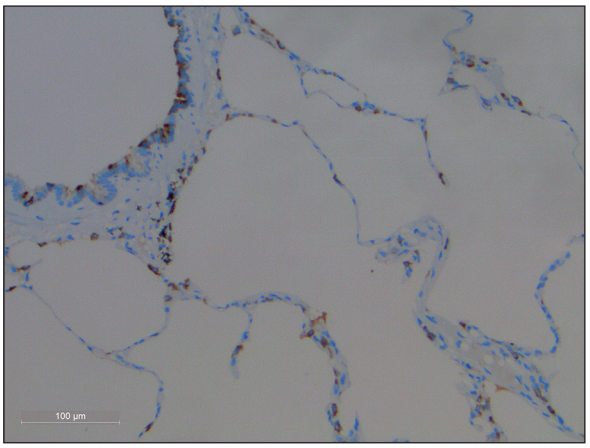

Figure 1. Surfactant Protein B in control normal lungs (20×): positivity in alveolar type II cells; focal positivity in some bronchial cells (top left).

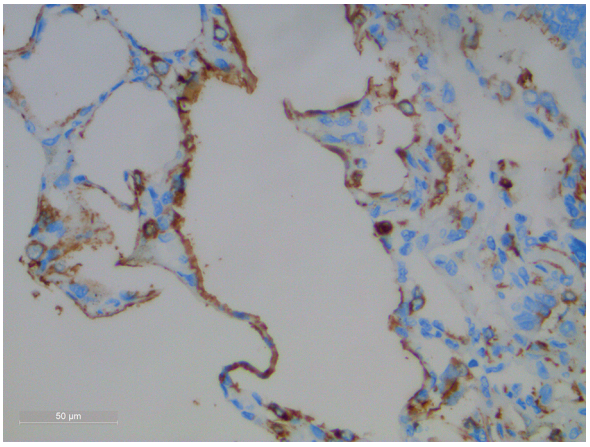

Figure 3. Surfactant Protein A in control normal lungs (40×): immunostaining for SP-A in alveolar type II cells; there is a thin layer of surfactant along the alveolar walls.

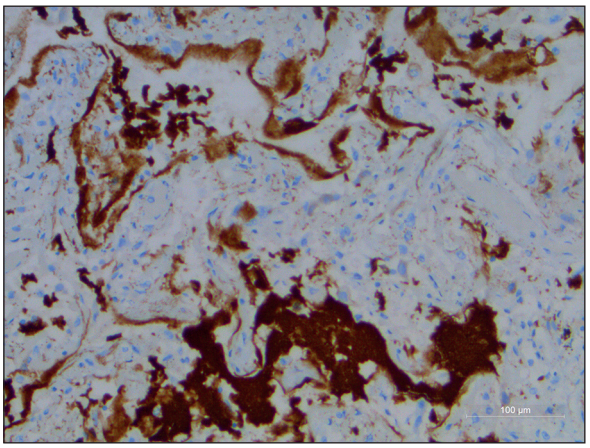

Figure 4. Surfactant Protein A in COVID-19 lungs (20×): immunostaining for SP-A shows abundant and condensed masses of SP-A within the alveolar spaces.

Islam and Khan[20], recently on lung transcriptome in COVID-19 demonstrated a downregulation of the surfactant proteins due to the viral infection.

Given that there are no validated therapies for ARDS following SARS-CoV-2 infection, finding new drugs and better alternatives for COVID-19 therapy represents a challenge for the scientific comunity[21]. Preliminary data on COVID-19 patients with severe hypoxia treated with intra-tracheal natural surfactant indicate exogenous surfactant as an alternative option. This was particularly evident in subjects with a critical derangement of lung function, in association with prone position, elevated Positive End-Expiratory Pressure (PEEP) or inhaled nitric oxide[22]. The principal bases for justifying the "surfactant approach" are the similarities between ARDS in COVID-19 patients and neonatal respiratory distress syndrome (NRDS), which is caused by surfactant deficiency[16]. Whereas in NRDS presenting in preterm newborns, surfactant deficiency is mainly due to immaturity of the lungs, in COVID-19, SARS-CoV-2 might impact the production and turnover of surfactant, inhibiting its delivery on the alveolar surface[18]. According with this hypothesis, AE2 cells should be considered the principal target of SARS-CoV-2[23], as previously found for SARS-Cov[24] and Influenza A virus[25].

Our immunohistochemical results confirm hyperplasia of the alveolar type II epithelial cells but also a marked increase in surfactant production in the lung of COVID patients with severe ARDS. The most significant sign of SP-A overproduction is the formation of large SP-A aggregates, often occupying the whole alveolar space. This surfactant is probably inactive or dysregulated.

Conclusions

Exogenous surfactant is a life-saving treatment for neonatal respiratory distress syndrome but in adults presenting with ARDS, this treatment is not always adeguate and satisfactory[6]. Surfactant administration may help to improve the clinical course in ARDS patients, but is not sufficient for patients affected COVID-19 ARDS[26].

Our data confirm at immunohistochemical level, that surfactant production is dysregulated in COVID 19 patients. The conseguences are well evidenced by the histological features, characterized by a reduction of alveolar spaces, often with atelectatic zones, associated with hyaline membrane formation. These feature contrast with a normal surfactant function. Moreover, the large amounts of surfactant produced by alveolar type II cells, giving rise to SP-A accumulation inside alveoli, could represent an obstacle to a significant benefit by exogenous surfactant therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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