International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (4): 106-112
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Review Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

# The Status of Surfactant as a Treatment Option for COVID19

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Received: 14 Jul 2020/ Accepted: 16 Aug 2020 / Published online: 1 Oct 2020
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## Abstract
Severe acute respiratory syndrome coronavirus 2 (SARS COV 2 virus) is known to cause coronavirus disease from December 2019 (COVID19). There is no approved treatment for the disease. The main standard of care is identification, isolation, contact tracing and observation. The main predominat organ affected is the lung. The main crucial factor in COVID 19 infection is binding of spike protein (S protein) of coronavirus to Angiotensin Converting Enzyme Type 2 (ACE2 receptor). ACE2 receptors are present in many cell types and tissues of the body including the lungs, heart, blood vessels, kidneys, liver and gastrointestinal tract. In the lungs, these are located on Type 2 pneumocytes. Type 2 pneumocytes are involved in the production of surfactant. Surfactant helps in lowering the surface tension in the lung and prevent it from collapse. Destruction of Type 2 pneumocytes in the lung in COVID19 is presumed to cause Acute Respiratory Distress Syndrome (ARDS), ARDs is characterized by presence of alveolar damage with serous and fibrin exudation, presence of hyaline membrane, presence of inflammatory infiltrate with predominant presence of monocytes and macrophages and alveolar thickening. Few clinical trials are ongoing in various stages to evaluate the use of surfactant therapy in COVID19 infection. This review article is on the current status of surfactants in the management of COVID-19 based on published literature obtained from PUBMED and studies from ClinicalTrials.gov listing.

**Keywords**
COVID 19, Surfactant, Angiotensin 2 receptor, ARDS.

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**INTRODUCTION:**
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus causing Coronavirus Disease 2019 (COVID-19) can cause lung complications such as pneumonia and, in the most severe cases, Acute Respiratory Distress Syndrome (ARDS). Sepsis, another possible complication of COVID-19, can also cause lasting harm to the lungs and other organs. There is no approved treatment for the disease. The main standard of care is identification, isolation, contact tracing and observation. [1] The pathological features observed in the COVID 19 patient lung looks similar to the ARDS lung injury. There has been presence of alveolar damage with serous exudation and fibrin exudation, hyaline membrane formation, and presence of inflammatory infiltrate mostly predominate by macrophages and monocytes. There is also presence of multinucleated giant cells, desquamation of alveolar epithelium and thickening of alveolar septum. [2] Many treatment options are being tried for COVID 19, but till date we do not have any specific antiviral therapies. Thus, supportive and symptomatic management is being provided to infected patients.
SARS-CoV-2 enters the cells through the Angiotensin Converting Enzyme Receptor 2 (ACE2). As they enter

through the respiratory tract, SARS-CoV-2 may specifically destroy cells, which predominantly express the ACE2 receptor on their surfaces. In the respiratory tract, they may destroy the type II alveolar cells. Type II alveolar cells are the "defender of the alveolus" and are the progenitor cells for the alveolar epithelium. They maintain alveolar homeostasis, and control the inflammatory response especially after microbial lung damage. They produce protective lung surfactant. Surfactant reduces the lung surface tension and facilitates breathing. It also enhances gas exchange, are initiates repair processes after trauma. It is believed that ACE2 itself protects from lung injury though anti-inflammatory and anti-fibrotic mechanisms. [3]

Recently, there are some publications that looked into the role of surfactant therapy for ARDS in COVID-19. Few studies are in also in clinical trial phase. [4] With this background, this review was undertaken to evaluate the current status of surfactant in the management of COVID-19.

**Evolution of Surfactant**

Surfactant was first discovered by a German - born physiologist, Kurt von Neergaard, in late 1920s in Switzerland. He discovered the function of pulmonary surfactant in increasing the compliance of lung by reducing the surface tension. [5] In late 1950s Richard pattle, a physicist working on nerve gases in England stated that the absence of lining in premature infants lung may be responsible for respiratory distress.[6] In 1959 Avery and Mead discovered the relationship between surfactant deficiency and RDS seen in preterm Infants. In 1980, Fujiwara et al performed treatment via the respiratory tract in 10 pediatric patients with RDS, using an artificial Surfactant extracted from bovine lung. This was the first successful treatment using artificial surfactant supplementation in humans, and has since resulted in improvement in oxygenation, treatment progress, and prognosis. [7]

**SURFACTANT AND ITS ROLE IN THE LUNG**

Pulmonary surfactant, is a complex material that lines the alveolar surface of the lung, is synthesized in the type II Pneumocytes and stored in the lamellar bodies. Surfactant consists largely of phospholipids, of which phosphatidylcholine (68%) is by far the most abundant component, and is mainly responsible for surface activity. Surfactant also contains four unique proteins, surfactant protein (SP)-A, SP-B, SP-C, and SP-D, which are synthesized in a lung-specific manner. Synthesis of surfactant lipids and proteins is developmentally regulated in fetal lung and can be accelerated by glucocorticoids and other hormones [8]. By reducing alveolar surface tension, pulmonary surfactant stabilizes the alveoli and prevents them from collapse. Alterations to the pulmonary surfactant system have long been implicated in the course of inflammatory lung diseases such as the Acute Respiratory Distress Syndrome (ARDS) [9]. Surfactant also serves as a barrier to pathogens improves mucociliary transport helps limit the development of high-surface-tension pulmonary edema and inhibits leakage of serum components into the airway [10].

**DIFFERENT TYPES OF SURFACTANT:**

**Natural surfactant:**

Natural surfactants are generally derived from either lung lavage or minced lung of bovine or porcine origin. Some of the examples are Curosurf, Survanta, Bovine lipid extract surfactant (BLES). [11]Natural surfactant preparations contain variable amounts of SP-B and SP-C that enhance surface tension-lowering properties and are preferred above synthetic preparations. [12]

**Synthetic surfactant:**

The basis of these preparations is a highly simplified phospholipid mixture. Some of them are Venticute, which contains recombinant SP-C (rSP-C), Ucinactant (Surfaxin), is characterized by the addition of KL4 (sinapultide), a non-natural polypeptide that was designed to resemble SP-B. These contain surfactant protein like additives which are synthesised majorly by recombinant technology. [11]

**Clinical condition where surfactant therapy is approved already or are under investigation (other than COVID-19) is mentioned in Table 1**

| S.NO | Clinical Application | PATHOPHYSIOLOGY | REFERENCE |
|---|---|---|---|
| 1 | Respiratory distress syndrome (RDS) | RDS is the most common cause of Respiratory failure in preterm infants, especially those born less than30 weeks of gestation due to less surfactant in the lung.[13] | Gitlin JD, et al performed a randomized prospective uncontrolled study in the year 1987, to know the effect of bovine surfactant in 41 low birth weight infants diagnosed with hyaline membrane disease, The study showed there is |

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|---|---|---|---|
| | | | significant improvement in oxygenation within 4 hrs of administration of surfactant.[14] |
| 2 | Acute Respiratory Distress Syndrome | In ARDS,surfactant inactivation or dysfunction occurs due to inflammation.Major mechanisms of surfactant inactivation in ARDS include decreased synthesis by the type II pneumocytes, or direct inhibition of surfactant function by substances like blood, serum proteins or proteinaceous edema fluid that occur due to lung injury.[15] | T.J Gregory et al, performed a randomized, Prospective control open label clinical study,to analyze safety and efficacy of bovine surfactant in Patients with ARDS in the year 1997. Subjects in the surfactant group showed mortality 18.8% as compared to control group mortality of 43.8%. [16]<br>Walmarth et al conducted a multicentric non control study in the year 2002 to analyze the effect of surfactant in ARDS and septic shock. They stated that Bronchoscopic instillation high dose of natural Bovine surfactant increased arterial oxygenation ,resulting in improvement of gaseous exchange.[17] |
| 3 | Meconium Aspiration Syndrome | Aspiration of meconium causes mechanical obstruction of airways, chemical pneumonitis and inactivation of surfactant. The inhibitory effect of meconium is dose dependent.[18] | A randomized controlled study was conducted by R D Findlay et al, in the year 1995 to determine the effect of high-dose surfactant therapy,in morbidity of term infants ventilated for meconium aspiration syndrome.Surfactant therapy when started within 6 hrs of therapy in meconium aspiration syndrome showed improved oxygenation.[18] |
| 4 | Pneumonia | Neonatal pneumonia caused by group B streptococci may manifest as RDS, because endogenous surfactant could become inactivated by the inflammatory exudate. Several babies with neonatal group B streptococcus pneumonia who have received exogenous surfactant Curosurf showed improved lung function.[19] | A Prospective Cohort Study was conducted by Deshpande et al, to evaluate whether exogenous surfactant therapy improves oxygenation and gas exchange in late preterm and term neonates with early onset pneumonia and respiratory failure.Neonates were all requiring mechanical ventilation and they received Surfactant therapy, Results showed, there is significant improvement in oxygenation in preterm and term neonates after surfactant therapy. This study showed there is substanstial role of Surfactant in the treatment of early onset Pneumonia.[20] |
| 5 | Lung hypoplasia | The lungs of neonates with congenital diaphragmatic hernia (CDH) are not only small but also biochemically immature.So the production of surfactant is inadequate leading to respiratory distress. | Studies on experimental CDH induced in lambs by fetal surgery by Hratch et al, in the year 1995, indicated that the hypoplastic lung stability might be improved by treatment with exogenous surfactant.[21] |

**COVID-19**

Coronavirus disease is caused by SARS Cov-2 virus. The binding of viral spike protein to the ACE 2 receptor is the main crucial part of coronavirus infection. ACE2 receptor is mainly expressed in the type 2 alveolar cells in the lung. ACE 2 converts angiotensin 2 to angiotensin 1 & 7. Angiotensin1-7 interacts with MassR G-protein coupled receptor ,which leads to cellular signaling and further causing cellular protective effects like vasodilation, anti - atrophy .[22,23] Binding of RBD spike of virus to ACE2 receptor causes down expression of receptor & also damage to the type2 alveolar cells which produce surfactant.[24] The Downregulation of ACE2 receptor leads to the Activation of RAAS leading to vasoconstriction and development of severe acute respiratory distress syndrome or acute lung injury.[25] So there is deficiency of Surfactant production due to the damage to the type 2 Alveolar cells in the lung in coronavirus disease.

In a Retrospective review study done by Pan F et al, in Wuhan China, There main aim is to note the radiological changes in COVID19 patients .In patients without severe respiratory disease, the major pulmonary CT findings of COVID-19 were ground-glass opacities(GGO), crazy-paving pattern, and consolidation predominantly in subpleural locations in the lower lobes, The typical mild COVID-19 mainly starts as small subpleural unilateral or bilateral Ground glass opacities in the lower lobes, which then develops into the crazy-paving pattern and subsequent consolidation. After more than 2 weeks, the lesions are gradually absorbed with residual GGO and subpleural parenchymal bands. [26]

In a case report by junziang lee et al, stated, a 33 year old woman with coronavirus disease, confirmed by fluorescence polymerase chain reaction, her unenhanced chest CT of lungs showed multiple peripheral ground glass opacities in both the lungs without sparing the subpleural regions. [27]

A two center descriptive study was conducted by Carsana et al, in Italy to evaluate the lung postmartum changes in COVID19 patients. All cases showed features of both exudative and proliferative phase of alveolar damage, capillary congestion, necrosis of pneumocytes, presence of hyaline membrane, intraaalveolar edema, type 2 pneumocyte hyperplasia was seen almost in every case, squamous metaplasia with atypia and platelet - fibrin thrombi, The inflammatory infiltrates are largely composed of macrophages in alveolar lumina and lymphocytes in the interstitium, diffuse alveolar damage was predominant in COVID infection resembling the lung in severe acute respiratory disease. There is presence of platelet- fibrin thrombi in small arterial vessels. [28]

A post martum biopsy was conducted by Sufan T et al, on four patients who died of coronavirus disease. All four patients who died had underlying immunocompromised condition like chronic lymphocytic leukaemia, hypertension, diabetes, cirrhosis. Post martum changes were observed in the lung, liver, heart. In the lung there was diffuse alveolar damage, hyaline membrane formation, and hyperplasia of type 2 pneumocytes. Consolidation by fibroblastic proliferation with extracellular matrix and fibrin forming clusters in airspaces is evident. In the liver, there was mild lobular infiltration by small lymphocytes, and centrilobular sinusoidal dilation. Heart showed mild focal fibrosis with hypertrophy. They have stated that changes in the liver and heart may be secondary to COVID 19 or due to underlying immunocompromised conditions. [29]

Lisa M et al, have conducted autopsies in two COVID positive patients, changes like diffuse alveolar damage, and airway inflammation, and bronchopneumonia are noted in lung .[30] The above study findings in Lungs in COVID19 patients resemble the lung findings in ARDS patients.

Although surfactant alterations are implicated in the pathogenesis of ARDS, surfactant replacement showed no proven benefit in clinical trials conducted. Successful outcome of surfactant therapy in ARDS depend on many factors such as etiology, Timing of surfactant, mode of administration, dose of surfactant, and type of surfactant. [31]

The treatment of COVID19 related ARDS lung injury by Surfactant therapy, may involve various factors, such as

- Timing of surfactant application, i.e. Surfactant therapy when given as early as possible may yield good results.[18]
- Mode or Route of Administration, Previous studies have shown that surfactant therapy through bronchoscopic instillation have shown more positive results when compared to Surfactant therapy when given by Aerosol nebulization in ARDS patients.[32,33]
- Dose of the Surfactant is one of the important factor, Surfactant therapy when given in large dose through bronchoscopic instillation have yielded good results.[34]
- Type of Surfactant also plays a Crucial Role, In a study conducted by Gunther et al, to investigate the impact of bronchoscopic surfactant administration, on the biochemical and biophysical surfactant properties in patients with severe and early acute respiratory distress syndrome (ARDS) and Septic shock 27 patients

received 300-500 mg·kg·body·weight of a natural bovine surfactant extract (Alveofact®) through a flexible bronchoscope. Broncho alveolar lavages were performed 3 h prior to, and 15–18 h and 72 h after surfactant administration. Surfactant treatment resulted in a marked increase in the lavagable phospholipid (PL) pool, complete normalization of the Phospholipid profile, and its SP-B and SP-C content as well as the fatty acid composition of the phosphatidylcholine class was noted. Surface tension lowering properties significantly improved. This study Suggest that Bronchoscopic instillation of large quantities of natural surfactant causes far reaching restoration of biochemical surfactant. Properties and significant improvement in COVID 19 patients. [34] Natural Surfactant preparations containing surfactant proteins like B&C are said to act more rapidly when compared to synthetic surfactant which are surfactant protein free. Natural surfactant are said to reduce neonatal mortality more when compared to synthetic surfactant. [35]

**Below are some Surfactant clinical trials registered in Clinical trails.org which are mentioned in Table 2.**

| S.NO | STUDY TITLE | INTERVENTION | SPONSOR | STATUS |
|---|---|---|---|---|
| 1 | Exogenous Surfactant Administration For patients with COVID19 | Bovine Lipid Extract | Lawson Health Research Institute, London | Not recruiting as of June 11 2020 |
| 2 | Randomized controlled Phase 2 trial of Poractant Alpha by fibreoptic Bronchoscopy directed endobronchial administration in Acute Respiratory distress Syndrome due to COVID19 viral Pneumonia | Poractant alpha (Curosurf) | Lenclud versalis Hospital, France | Not recruiting as of May12,2020 |
| 3 | A clinical Trail of nebulized surfactant for the treatment of Moderate to Severe COVID19 | Covsurf | University Hospital South Hampton NHS foundation Trust. Bill and Melinda Gates Foundation. University College, London | Not recruiting as of May 28 2020 |
| 4 | A multi center, Single Treatment Study to assess the safety and efficacy of Lyophilized Lucinactant in Patients with COVID19 associated Lung injury | Lucinactant,$KL_4$/ | Wind tree Therapeutics | Not recruiting as of May 15 2020 |

Total of 16 articles were obtained when "SURFACTANT COVID19" search term was given in Pubmed. Out of the 16, only 3 articles were related to surfactant therapy in COVID19.

Koumbourlis et al, have stated that COVID19 changes in the Lung resemble Respiratory Distress Syndrome in neonates. They stated that exogenous surfactant should start as soon as possible in the course of disease, and outcome depends on several factors such as Dose, Frequency and Mode of Administration. [36]

Urusula M et al, Have stated that the, meconium aspiration syndrome in neonates resemble COVID19 due to deficiency of surfactant and destruction of type 2 alveolar cells, They stated that early administration of Natural Surfactant along with current standards of ARDS intensive care treatment in COVID 19 pneumonia patients may yield better results.[37]

Balaji AP et al, Have mentioned, depletion of pulmonary surfactants causes strong acute respiratory distress syndrome, they have proposed a remedy which mainly focuses on Target specific action, here red blood cells (RBCs) as a conveyor with embedded artificial surfactant and protruding ACE2 receptors for the target-specific delivery. [38]

**CONCLUSION:**

Coronavirus disease is very rapidly spreading global pandemic. Lung damage remains the most predominant organ affected due to COVID19. Understanding the pathogenesis and pathology in the lung will help in the diagnosis and management of COVID 19. [39] Many clinical trials are underway for the development of vaccine to Coronavirus. Some drugs have been approved by different countries according to their respective protocols. But the definite treatment still remains a big challenge. Surfactant therapy was success full in treating infants with respiratory distress syndrome till now. Based on the previous articles, Pathology changes in the lung due to COVID19 resemble ARDS lung injury. Based on our review of the previous articles on surfactant, we conclude by saying surfactant can be considered as an option for the treatment of COVID19 along with other drugs.

**REFERENCES:**

1. Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, Skipper CP, Nascene AA, Nicol MR, Abassi M, Engen NW. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. *New England Journal of Medicine. (2020)* Jun 3.
2. Yao XH, Li TY, He ZC, Ping YF, Liu HW, Yu SC, Mou HM, Wang LH, Zhang HR, Fu WJ, Luo T. A pathological report of three COVID-19 cases by minimally invasive autopsies. Zhonghua bing li xue za zhi= *Chinese journal of pathology. (2020)* Mar 15; 49:E009.
3. Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. *Annual Review of Pathology: Mechanisms of Disease. (2011)* Feb 28; 6:147-63.
4. Lutz C, Maher L, Lee C, Kang W. COVID-19 preclinical models: human angiotensin-converting enzyme 2 transgenic mice. *Human Genomics. (2020)* Dec; 14:1-1.
5. Sardesai S, Biniwale M, Wertheimer F, Garingo A, Ramanathan R. Evolution of surfactant therapy for respiratory distress syndrome: past, present, and future. *Pediatric research. (2017)* Jan; 81(1):240-8.
6. Halliday HL. Surfactants: past, present and future. *Journal of perinatology. (2008)* May; 28(1):S47-56.
7. Soll R, Blanco F. Natural surfactant extract versus synthetic surfactant for neonatal respiratory distress syndrome. *Cochrane Database of Systematic Reviews. (2001)*(2).
8. Rooney SA, Young SL, Mendelson CR. Molecular and cellular processing of lung surfactant 1. *The FASEB Journal. (1994)* Sep; 8(12):957-67.
9. Schmidt R, Markart P, Ruppert C, Wygrecka M, Kuchenbuch T, Walmrath D, Seeger W, Guenther A. Time-dependent changes in pulmonary surfactant function and composition in acute respiratory distress syndrome due to pneumonia or aspiration. *Respiratory research. (2007)* Dec 1; 8(1):55.
10. Glasser JR, Mallampalli RK. Surfactant and its role in the pathobiology of pulmonary infection. *Microbes and infection. (2012)* Jan 1; 14(1):17-25.
11. Been JV, Zimmermann LJ. What's new in surfactant? *European journal of pediatrics. (2007)* Sep 1; 166(9):889-99.
12. Auten RL, Notter RH, Kendig JW, Davis JM, Shapiro DL. Surfactant treatment of full-term newborns with respiratory failure. *Pediatrics. (1991)* Jan 1; 87(1):101-7.
13. Ramanathan R. Choosing a right surfactant for respiratory distress syndrome treatment. *Neonatology. (2009)*; 95(1):1-5.
14. Davidson WJ, Dorscheid D, Spragg R, Schulzer M, Mak E, Ayas NT. Exogenous pulmonary surfactant for the treatment of adult patients with acute respiratory distress syndrome: results of a meta-analysis. *Critical Care. (2006)* Apr 1; 10(2):R41.
15. Gregory TJ, Steinberg KP, Spragg RO, Gadek JE, Hyers TM, Longmore WJ, Moxley MA, Cai GZ, Hite RD, Smith RM, Hudson LD. Bovine surfactant therapy for patients with acute respiratory distress syndrome. *American journal of respiratory and critical care medicine. (1997)* Apr; 155(4):1309-15.
16. Walmrath D, Grimminger F, Pappert D, Knothe C, Obertacke U, Benzing A, Günther A, Schmehl T, Leuchte H, Seeger W. Bronchoscopic administration of bovine natural surfactant in ARDS and septic shock: impact on gas exchange and haemodynamics. *European Respiratory Journal. (2002)* May 1; 19(5):805-10.
17. Robertson B. New targets for surfactant replacement therapy: experimental and clinical aspects. *Archives of disease in childhood Fetal and neonatal edition. (1996)* Jul; 75(1):F1.
18. Findlay RD, Taeusch HW, Walther FJ. Surfactant replacement therapy for meconium aspiration syndrome. *Pediatrics. (1996)* Jan 1; 97(1):48-52.
19. Sherman MP, Campbell LA, Merritt TA, Long WA, Gunkel JH, Curstedt T, Robertson B. Effect of different surfactants on pulmonary group B streptococcal infection in premature rabbits. *The Journal of pediatrics. (1994)* Dec 1; 125(6):939-47.
20. Deshpande S, Suryawanshi P, Ahya K, Maheshwari R, Gupta S. Surfactant therapy for early onset pneumonia in late preterm and term neonates needing mechanical ventilation. *Journal of Clinical and Diagnostic Research: JCDR. (2017)* Aug; 11(8):SC09.
21. Wilcox DT, Glick PL, Karamanoukian H, Rossman J, Morin III FC, Holm BA. Pathophysiology of congenital diaphragmatic hernia. V. Effect of exogenous surfactant therapy on gas exchange and lung mechanics in the lamb congenital diaphragmatic hernia model. *The Journal of pediatrics. (1994)* Feb 1; 124(2):289-93.
22. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE. A novel angiotensin-converting enzyme–related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circulation research. (2000)* Sep 1; 87(5):e1-9.
23. Santos RA, Ferreira AJ, Simões e Silva AC. Recent advances in the angiotensin-converting enzyme 2–angiotensin (1–7)–Mas axis. *Experimental physiology. (2008)* May 1; 93(5):519-27.
24. Glowacka I, Bertram S, Herzog P, Pfefferle S, Steffen I, Muench MO, Simmons G, Hofmann H, Kuri T, Weber F, Eichler J. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. *Journal of virology. (2010)* Jan 15; 84(2):1198-205.
25. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA. *Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. (2005)* Jul; 436(7047):112-6.
26. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, Zheng D, Wang J, Hesketh RL, Yang L, Zheng C. Time course of lung changes on chest CT during recovery from 2019 novel coronavirus (COVID-19) pneumonia. *Radiology. (2020)* Feb 13:200370.
27. Lei J, Li J, Li X, Qi X. CT imaging of the 2019 novel coronavirus (2019-nCoV) pneumonia. *Radiology. (2020)* Apr; 295(1):18.
28. Carsana L, Sonzogni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P, Rech R, Colombo R, Antinori S, Corbellino M, Galli M. Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre

descriptive study. *The Lancet Infectious Diseases. (2020)* Jun 8.
29. Tian S, Xiong Y, Liu H, Niu L, Guo J, Liao M, Xiao SY. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Modern Pathology. (2020)* Apr 14:1-8.
30. Barton LM, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. Covid-19 autopsies, oklahoma, usa. *American Journal of Clinical Pathology. (2020)* May 5; 153(6):725-33.
31. Anzueto, A. Exogenous surfactant in acute respiratory distress syndrome: more is better. *(2002)*: 787-789.
32. Weg JG, Balk RA, Tharratt RS, Jenkinson SG, Shah JB, Zaccardelli D, Horton J, Pattishall EN, Albertson TE, Caldwell EJ, MacIntyre NR. Safety and potential efficacy of an aerosolized surfactant in human sepsis-induced adult respiratory distress syndrome. *Jama. (1994)* Nov 9; 272(18):1433-8.
33. Wiswell TE, Smith RM, Katz LB, Mastroianni L, Wong DY, Willms D, Heard S, Wilson M, Hite RD, Anzueto A, Revak SD. Bronchopulmonary segmental lavage with surfaxin (KL4-surfactant) for acute respiratory distress syndrome. *American journal of respiratory and critical care medicine. (1999)* Oct 1; 160(4):1188-95.
34. Günther A, Schmidt R, Harodt J, Schmehl T, Walmrath D, Ruppert C, Grimminger F, Seeger W. Bronchoscopic administration of bovine natural surfactant in ARDS and septic shock: impact on biophysical and biochemical surfactant properties. *European Respiratory Journal. (2002)* May 1; 19(5):797-804.
35. Halliday HL. Synthetic or natural surfactants. *Acta Pædiatrica. (1997)* Mar; 86(3):233-7.
36. Koumbourlis AC, Motoyama EK. Lung Mechanics in COVID-19 Resemble RDS not ARDS: Could Surfactant be a Treatment? *American Journal of Respiratory and Critical Care Medicine. (2020)* Jun 24(ja).
37. Mirastschijski U, Dembinski R, Maedler K. Lung Surfactant for Pulmonary Barrier Restoration in Patients with COVID-19 Pneumonia. *Frontiers in Medicine. (2020)* May 22; 7:254.
38. Balaji AP, Bhuvaneswari S, Kumar DN. Deceiving SARS-CoV-2 molecular-tropism clues–A combinational contemporary strategy. *Medical Hypotheses. (2020)* Jun 7:109976.
39. Jain A. COVID-19 and lung pathology. I*ndian Journal of Pathology and Microbiology. (2020)* Apr 1; 63(2):171.