# Presence of hyaluronan in lung alveoli in severe Covid-19: An opening for new treatment options?

Urban Hellman[1],*⍟, Mats G. Karlsson[2]⍟, Anna Engström-Laurent[1], Sara Cajander[3], Luiza Dorofte[2], Clas Ahlm[4], Claude Laurent[5]⍟, and Anders Blomberg[1]

From the Departments of [1]Public Health and Clinical Medicine, [4]Clinical Microbiology, and [5]Clinical Science, Umeå University, Umeå, Sweden and the Departments of [2]Laboratory Medicine and [3]Infectious Diseases, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

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**Severe coronavirus disease 2019 (Covid-19) is characterized by inflammation of the lungs with increasing respiratory impairment. In fatal Covid-19, lungs at autopsy have been filled with a clear liquid jelly. However, the nature of this finding has not yet been determined. The aim of the study was to demonstrate whether the lungs of fatal Covid-19 contain hyaluronan, as it is associated with inflammation and acute respiratory distress syndrome (ARDS) and may have the appearance of liquid jelly. Lung tissue obtained at autopsy from three deceased Covid-19 patients was processed for hyaluronan histochemistry using a direct staining method and compared with staining in normal lung tissue. Stainings confirmed that hyaluronan is obstructing alveoli with presence in exudate and plugs, as well as in thickened perialveolar interstitium. In contrast, normal lungs only showed hyaluronan in intact alveolar walls and perivascular tissue. This is the first study to confirm prominent hyaluronan exudates in the alveolar spaces of Covid-19 lungs, supporting the notion that the macromolecule is involved in ARDS caused by SARS-CoV-2. The present finding may open up new treatment options in severe Covid-19, aiming at reducing the presence and production of hyaluronan in the lungs.**

The ongoing pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has hitherto caused more than 730,000 reported deaths globally (by August 10, 2020, according to Johns Hopkins University). In severe Covid-19, there is a pronounced inflammatory response in the lungs resulting in respiratory failure and potential death, especially among individuals with predisposing chronic diseases or advanced age.

The glycosaminoglycan hyaluronan (HA) has recently been suggested to be a potential cause of fatalities in Covid-19 lung infection (1). The authors referred to findings at autopsies of deceased Covid-19 patients where the lungs were filled with a clear liquid jelly, much resembling the lungs of wet drowning (2). Although the nature of this liquid jelly has not been determined, HA has been proposed, as it is associated with acute respiratory distress syndrome (ARDS) (1, 3). Hyaluronan is an important component of all extracellular matrices, and its levels are generally elevated in tissue in response to inflammation and injury (4). The HA molecule is highly hygroscopic and

has the ability to absorb water up to 1,000 times its molecular weight, which can promote edema (5). Due to its high molecular weight and semiflexible polymer chain, it forms a gel-like fluid with high viscosity causing exclusion properties and barrier functions in tissues (5). There are also a large number of HA-binding proteins that can involve HA in diverse biological processes (6). High-molecular weight HA predominates in most tissues under healthy conditions, whereas fragmented low-molecular weight HA polymers predominate at sites of active inflammation (4).

It has been proposed that HA could be responsible for some of the disease manifestations and mortality in the most severe form of Covid-19 (1). However, arguments that HA is involved in the pathogenesis of Covid-19 must be verified before new treatment options against the formation of HA can be discussed. Accordingly, the aim of our study was to demonstrate that hyaluronan is present in alveolar spaces in lungs of deceased patients with severe Covid-19.

## Results

Autopsy lung tissue from the three Covid-19 cases is shown in Fig. 1 (magnification ×20). In two patients, histochemical staining revealed intra-alveolar and interstitial HA localization in the exudative phase (Fig. 1, A and B), whereas the third patient showed the proliferative phase of the Covid-19–associated diffuse alveolar damage (Fig. 1C).

The alveolar spaces were filled with exudate and alveolar plugs exhibiting pronounced HA staining. The alveolar walls were hyperplastic and damaged, and HA staining was evident in the thickened perialveolar interstitium (Fig. 2, B–D).

In contrast, HA staining was only visible in the alveolar walls and in perivascular tissue in normal lung tissue (Figs. 2A and 3A).

The specificity of the staining method for HA is shown in Fig. 3 (B and C). Treatment of the section with hyaluronidase from bovine testes (in Fig. 3C) effectively abolished the HA staining.

## Discussion

The pathogenesis of Covid-19 is not fully understood, and there is an urgent need for effective treatments to alleviate the respiratory failure and decrease the case fatality rate in severe disease. This is the first report verifying the presence of HA in the alveolar spaces in lung tissue from three lethal Covid-19
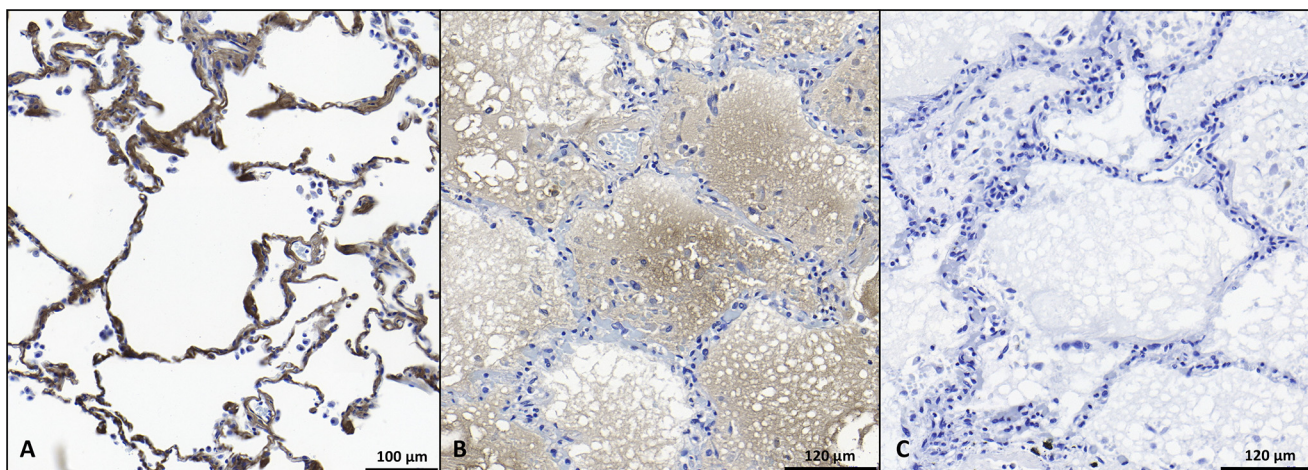
* For correspondence: Urban Hellman, urban.hellman@umu.se.
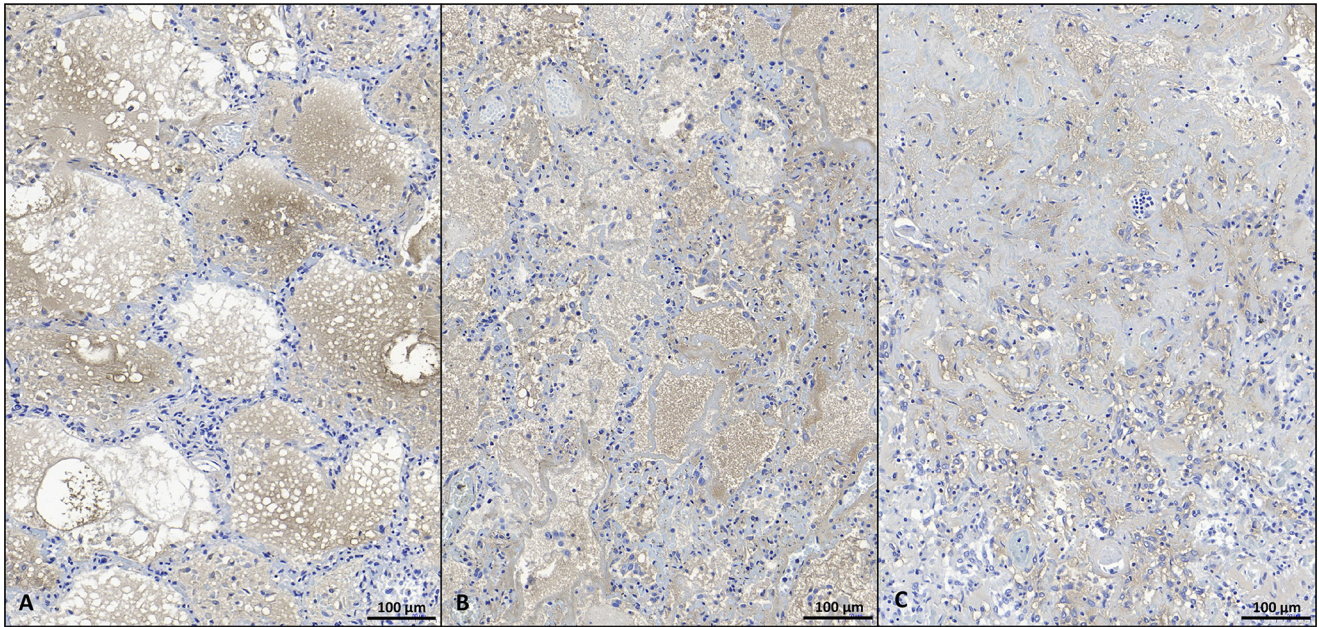
**Figure 1. Color light micrographs showing the staining pattern for HA as a brown precipitate in sections counterstained with Mayer's hematoxylin.** Autopsy lung tissue from the three Covid-19 cases (magnification ×20) showing intra-alveolar and interstitial HA staining in the exudative (*A* and *B*) and proliferative phase of the Covid-19–associated diffuse alveolar damage (*C*). *A* can be viewed in greater detail in Fig. 3*B* at higher magnification.
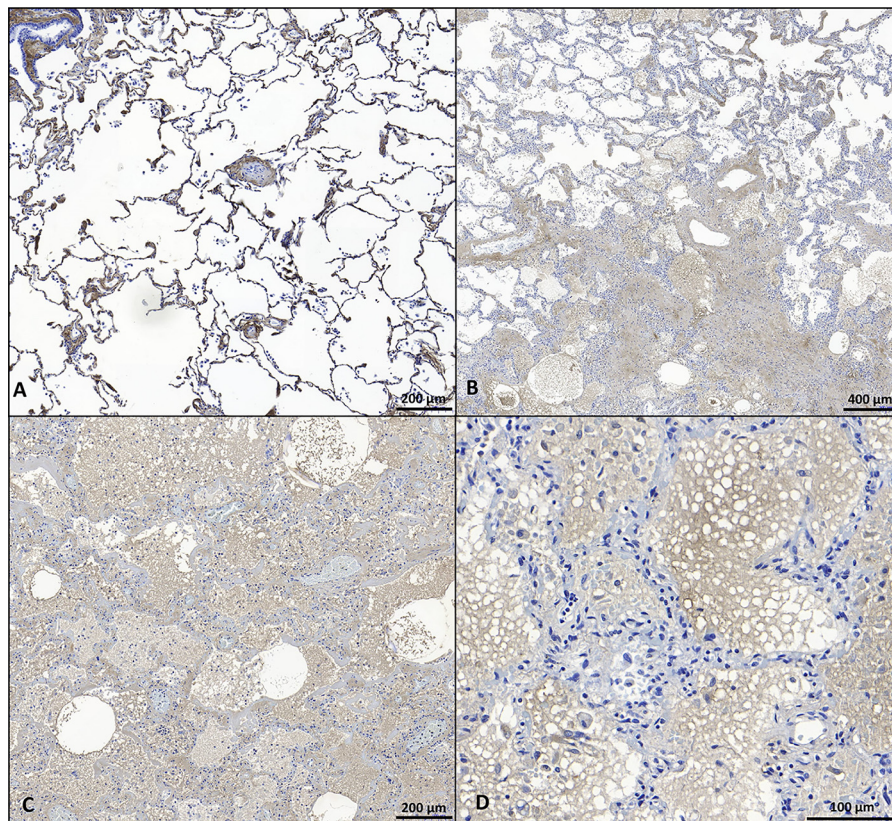


**Figure 2. Color light micrographs showing the staining pattern for HA as a brown precipitate in sections counterstained with Mayer's hematoxylin.** *A*, normal lung tissue showing HA positivity in alveolar walls and perivascular tissue (×10). *B*, overview (×5), showing the transition from less affected lung tissue without extensive alveolar exudate and plugs to areas with prominent HA positivity in the alveolar spaces from Covid-19 patients. *C* and *D*, intermediate- and high-power magnification (×10 and ×40) micrographs from two of the Covid-19 patients with extensive alveolar exudates.

**Figure 3. Color light micrographs showing the staining pattern for HA as a brown precipitate in sections counterstained with Mayer's hematoxylin.** *A*, normal lung tissue at ×40. *B* and *C*, Covid-19 lung tissue at ×40 showing the presence of HA in alveolar spaces (*B*) and the negative control slide pretreated with bovine testes hyaluronidase (*C*) from the same tissue block, showing the specificity of the reaction. The larger area of *B* can be viewed in Fig. 1A at lower magnification.

cases. In recent publications of post-mortem findings in Covid-19 lungs (2, 7), the authors describe some characteristic hallmarks, including hyaline membranes, intra-alveolar fibrinous exudate, and alveolar plugs. In our material, the intra-alveolar material and perialveolar interstitium showed pronounced staining for HA, supporting the notion that HA plays an important role in the pathogenesis of severe Covid-19.

There is accumulating evidence suggesting that suppressed innate antiviral defenses along with a hyperactive and dysregulated innate immune response may contribute to a "cytokine storm" that drives the clinical presentation of acute lung injury in patients with severe Covid-19 (1, 8–11). Several important inflammatory cytokines, such as TNF$\alpha$, IL-1$\beta$, and IL-6, are involved in severe Covid-19 (12). The innate immune response, in terms of the antiviral interferon type I and III, has been shown to inhibit SAR-CoV-2 infections in cell lines (13). Furthermore, it has been shown that the increase in IL-6 in Covid-19–infected patients is associated with the need for mechanical ventilation due to primary respiratory failure and, eventually, with mortality in the disease (12, 14). A recent study has suggested that levels of HA in serum at admission could distinguish critically ill patients with Covid-19 infection (15). However, increased serum HA levels in Covid-19 patients do not necessarily mean that lung alveoli are filled with HA. Also, previous reports have suggested that a decline in peripheral blood lymphocytes, as well as an increase in peripheral inflammatory factors, such as CRP and IL-6, may be early warning indicators of a poor prognosis for Covid-19 patients (16).

The "cytokine storm," with high levels of inflammatory cytokines (IL-1 $\beta$, IL-6, and TNF$\alpha$) in the lungs of patients with severe Covid-19, implies a close relation to HA, as these cytokines are strong inducers of HA synthase 2 (HAS2) in endothelium, lung alveolar epithelial cells, and fibroblasts (17). This fits well with the notion that the state of "hyperinflammation" induces the production and accumulation of HA within the alveolar spaces of patients with severe Covid-19. It has also been shown that HA accumulates in the airways during influenza infection in mice and that HA may be a target for treatment (18).

In a recently published study, it was reported that in patients hospitalized due to severe Covid-19 and receiving either invasive mechanical ventilation or oxygen, anti-inflammatory treatment with dexamethasone resulted in lower 28-day mortality (19). Whereas corticosteroids in high doses may be more harmful than helpful, beneficial effects of corticosteroid treatment in Covid-19 are suggested to depend on the right dose, at the right time, and in the right patient (19). In connection with this, it is also interesting to note that corticosteroids are known to be effective in reducing HA levels in bronchoalveolar lavage and circulation (20, 21).

The verification of abundant HA in the lungs and, specifically, in alveolar spaces of individuals deceased with the most severe form of Covid-19 thus indicates a possible pathogenetic mechanism behind the hypoxemia and respiratory failure seen in these critically ill patients. A recent synopsis proposed some new possible approaches to the treatment of critically ill Covid-19 patients, among them reducing the presence, or inhibiting the production, of HA in the lungs (1). However, the article presented no proof at all that HA was present in Covid-19 lungs. Nevertheless, it was suggested that airway inhalation of hyaluronidase would degrade and reduce HA. It is possible that early in the disease, when hypoxemia is developing, inhalation of hyaluronidase could possibly clear the hygroscopic macromolecule from the lungs and facilitate respiration and oxygenation. Experimentally, it has been shown that intranasal administration of exogenous hyaluronidase can reduce lung HA content and restore lung function following influenza infection (18). Hyaluronan shows a molecular weight–dependent role in regulating inflammation, and HA fragments generated in the course of inflammation might influence the release of inflammatory cytokines (22). In inflammatory diseases, high-molecular weight HA can also be found in cross-linked complexes (*e.g.* with inter-$\alpha$-trypsin inhibitor heavy chain and versican) (22, 23). Regardless of size, a degradation of HA by hyaluronidase to smaller oligosaccharide polymers could therefore possibly reduce the ongoing inflammatory reaction in Covid-19.

Another suggested option is the clinical use of 4-methylum-belliferone (4-MU) (1), which can inhibit the production of HA in inflammation, autoimmunity, and cancer (4). 4-MU both inhibits the gene expression of two HA synthases (*HAS2* and *HAS3*) and blocks the last stage in the formation of HA from glucose metabolites (24). This substance or its chemical derivatives is already used in Chinese medicine, which may explain the observed positive effects of combined herbal therapies in some Covid-19 patients (1). In clinical trials, however, not including Covid-19 patients, 4-MU has been demonstrated safe during short-term administration in approved doses (4).

In conclusion, we have for the first time demonstrated a striking presence of hyaluronan in alveolar spaces of the lungs in lethal cases of Covid-19. Based on this novel finding, adjuvant treatment targeting hyaluronan may be a promising approach to reduce mortality in critically ill Covid-19 patients. However, clinical randomized trials are warranted to evaluate the safety and efficacy of these substances in the case of severe Covid-19.

## Experimental procedures

Lung tissue was obtained at autopsy from three adult Covid-19 positive patients, two males aged 47 and 48 years and one female aged 73 years. Two of the patients had been treated in the intensive care unit, and both of them showed signs of hyperinflammation. For comparison, normal lung tissue was obtained from four patients undergoing thoracic surgery, and the samples were processed in an identical way as the pathological Covid-19 lung tissue.

Formaldehyde-fixed (4%) and paraffin-embedded specimens of lung tissue were further processed for HA histochemistry using a direct and specific HA staining method (25). Sections of lung tissue were incubated with a hyaluronan-binding protein probe, donated by Corgenix (Broomfield, CO, USA). After incubation with Vectastain-Elite Avidin-Biotin complex reagent (Vector Laboratories, Burlingame, CA, USA), the HA stainings were developed in a solution of 3,3′-diaminobenzidine (Vector Laboratories). The sections were counterstained with Mayer's hematoxylin. Control slides were preincubated with hyaluronidase from bovine testes (Sigma), a selective HA-digesting enzyme, showing the specificity of the method. All slides were stained simultaneously in the same batch.

Ethical approval for the study was obtained from the Swedish Ethical Review Authority, Dnr. 2020-02204 and 2014/395-31. The ethical approval only allowed for limited clinical information at the time.

## Data availability

All data are contained within the article.

*Abbreviations*—The abbreviations used are: HA, hyaluronan; ARDS, acute respiratory distress syndrome; TNF, tumor necrosis factor; IL, interleukin; 4-MU, 4-methylumbelliferone.

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