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Hypoalbuminemia in COVID-19: assessing the hypothesis for underlying pulmonary capillary leakage

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Abstract. Wu MA, Fossali T, Pandolfi L, Carsana L, Ottolina D, Frangipane V, Rech R, Tosoni A, Lopez G, Agarossi A, Cogliati C, Meloni F, Marchini B, Nebuloni M, Catena E, Colombo R (University of Milan, Milan; IRCCS Policlinico San Matteo Foundation, Pavia; University of Milan, Milan; IRCCS Policlinico San Matteo Foundation, Pavia; University of Pavia, Pavia; University of Milan, Milan, Italy). Hypoalbuminemia in COVID-19: assessing the hypothesis for underlying pulmonary capillary leakage. *J Intern Med* 2021; https://doi.org/10.1111/joim.13208

Background. Since the first observations of patients with COVID-19, significant hypoalbuminaemia was detected. Its causes have not been investigated yet.

Objective. We hypothesized that pulmonary capillary leakage affects the severity of respiratory failure, causing a shift of fluids and proteins through the epithelial–endothelial barrier.

Methods. One hundred seventy-four COVID-19 patients with respiratory symptoms, 92 admitted to the intermediate medicine ward (IMW) and 82 to the intensive care unit (ICU) at Luigi Sacco Hospital in Milan, were studied.

Results. Baseline characteristics at admission were considered. Proteins, interleukin 8 (IL-8) and interleukin 10 (IL-10) in bronchoalveolar lavage fluid (BALF) were analysed in 26 ICU patients. In addition, ten autopsy ultrastructural lung studies were performed in patients with COVID-19 and compared with postmortem findings in a control group (bacterial pneumonia-ARDS and H1N1-ARDS). ICU patients had lower serum albumin than IMW patients [20 (18–23) vs 28 (24–33) g L^{-1}, $P < 0.001$]. Serum albumin was lower in more compromised groups (lower PaO_2-to-FiO_2 ratio and worst chest X-ray findings) and was associated with 30 days of probability of survival. Protein concentration was correlated with IL-8 and IL-10 levels in BALF. Electron microscopy examinations of eight out of ten COVID-19 lung tissues showed loosening of junctional complexes, quantitatively more pronounced than in controls, and direct viral infection of type 2 pneumocytes and endothelial cells.

Conclusion. Hypoalbuminaemia may serve as severity marker of epithelial–endothelial damage in patients with COVID-19. There are clues that pulmonary capillary leak syndrome plays a key role in the pathogenesis of COVID-19 and might be a potential therapeutic target.

Keywords: capillary leakage, capillary permeability, COVID-19, critical care, hypoalbuminaemia, SARS-CoV-2.

Introduction

The outbreak of the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection begun in December 2019 in Wuhan, China [1], rapidly spread to every continent, leading the scientific community to conduct multidisciplinary studies to investigate its pathogenesis and address issues related to the most appropriate management.

SARS-CoV-2 disease (COVID-19) usually progresses through subsequent phases (early infection with viral response phase, pulmonary phase

and hyperinflammation phase) [2]. In its most severe presentation, COVID-19 induces progressive hypoxemic respiratory failure ranging from mild pneumonia to acute respiratory distress syndrome (ARDS), mostly due to a dysregulated inflammatory response [3, 4]. During the hyperinflammation stage, many pro-inflammatory cytokines, chemokines and mediators (such as interleukin 6, interleukin 1β, tumour necrosis factor-α, granulocyte-colony stimulating factor, macrophage inflammatory proteins 1-α) have been shown to be significantly increased in peripheral blood, likely playing a pivotal role not only in respiratory injury but also in multiple organ failure. These molecules cause remarkable damage especially to those sites with high expression of angiotensin-converting enzyme 2 (ACE2), the functional receptor for SARS-CoV-2 cell entry, leading to the clinical picture of the so-called 'viral sepsis' [5, 6].

Thanks to autopsy studies, direct viral damage was also confirmed by the identification of viral particles in the bronchial and type 2 alveolar epithelial cells by electron microscopy [7,8].

Recently, interest has been raised on the role of endothelial dysfunction in COVID-19. The dysfunction appears to be the result of multiple concomitant mechanisms, including reaction to the invading pathogen as well as to hypoxia, activation and recruitment of immune cells (mononuclear cells and neutrophils), with the production of inflammatory mediators (including cytokines, polyphosphates, neutrophil extracellular traps – NETS) [9], and damage to cells releasing histones [10]. These hits confer to the vascular endothelium a procoagulant vasoconstriction-prone inflammatory status [11, 12]. The involvement of endothelial cells across vascular beds of different organs in a series of patients with COVID-19 has been shown, with the presence of viral elements within endothelial cells inducing cell death of both endothelial and immune cells (apoptosis, NETosis) [13]. This process may lead to the loss of integrity of the epithelial–endothelial (air-blood) barrier, with exudate in the alveolar cavity, a mechanism which is consistent with imaging findings, showing signs of interstitial-alveolar damage (B lines, white lung and patchy pattern at lung ultrasound, ground-glass opacities and hazy areas with slightly increased density at CT scan) [14, 15]. The nature of the above-mentioned alveolar exudate has been a matter of investigation.

Since our very first observations of patients with COVID-19 in the last days of February 2020, we found significant hypoalbuminaemia, both in patients admitted to intermediate care wards (IMWs, Internal and Emergency Medicine Departments) and in those rapidly admitted to intensive care units (ICUs). No proteinuria or protein-losing enteropathy of such an entity to justify such a significant hypoalbuminaemia was ever detected. Remarkably, the administration of albumin did not seem to be able to restore intravascular albumin concentration.

We conducted this multidisciplinary study to investigate the hypothesis that COVID-19 is able to induce pulmonary capillary leakage which, despite being secondary to the hyperinflammatory state and the cytokine storm, significantly contributes to the pathogenesis of COVID-19-related clinical picture.

Materials and methods

Study design and participants

This is a retrospective, observational cohort study carried out at Luigi Sacco Hospital, a referral centre for highly transmissible diseases in Milan, Italy, during the large COVID-19 epidemic surge in Northern Italy. Hospital charts of all patients ≥ 18 years of age admitted to the ICU and IMW between 21 February and 15 April 2020 were screened. Inclusion criteria were SARS-CoV-2-induced lung disease confirmed by real-time PCR on throat swab samples and a serum albumin measurement within 72 hours from hospital admission. Exclusion criteria were age < 18 years and missing serum albumin measurements in the first 72 hours since hospital admission. We compared the clinical characteristics of ICU and IMW patients.

Research and data collection protocols were approved by the Institutional Review Board (Comitato Etico di Area 1). Written informed consent was obtained by survivors and waived in all others.

For autopsies, the study followed the Italian general rules used for research related to scientific purposes (official regulations n.72 - 26/03/2012).

Procedures

Diagnostic and therapeutic interventions were chosen by the treating physician according to

current clinical practice. Patients were admitted to ICU whether they failed a trial with continuous positive airway pressure by a helmet and whether no do-not-resuscitate orders were in place. Failure was defined as respiratory rate > 30 breaths per minute and PaO_2-to-FiO_2 ratio < 150, or respiratory acidosis with pH < 7.36 and $PaCO_2$ > 50 mmHg, or agitation, or confusion.

Demographic features, laboratory results on admission, need for organ support, as well as ICU and hospital length of stay (LOS), and outcomes were retrieved from hospital charts.

Chest X-rays (CXRs) performed at hospital admission were independently reviewed by two physicians with experience in respiratory medicine, and a CXR scoring system specifically designed for semiquantitative assessment of lung disease in COVID-19 (named Brixia score) was calculated [16]. This ranks the pulmonary involvement on an 18-point severity scale according to the extent and characteristics of lung infiltrates. Bronchoalveolar lavage was collected from mechanically ventilated ICU patients according to the clinical requirement through disposable bronchoscope aScope™ 4 (Ambu A/S, Baltorpbakken, Denmark). Samples were centrifuged at 400 g for 10 min at room temperature.

To quantify interleukin 8 (IL-8) and interleukin 10 (IL-10), we used SimpleStep ELISA® kit (Abcam, Cambridge, UK). Briefly, 50 μL of each sample was added into ELISA kit wells also adding 50 μL antibody cocktail. After 1 hour at room temperature on a plate shaker, three washes were done to eliminate the unbounded antibody. 100 μL of the substrate was incubated for 10 min in the dark at room temperature on a plate shaker, followed by 100 μL stop solution to read the absorbance at 450 nm. For IL-8 analyses, samples were diluted (from 1:10 to 1:1000).

To quantify proteins in BALF samples, Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, MA, USA) was used. The standard range used was from 20 to 2000 μg mL^{-1}. 25 μL of each sample was added to a 96-well plate and 200 μL of BCA working reagent. After 30 min at 37°C, absorbance was read at 550 nm.

We collected lung tissues of ten patients with COVID-19 who died in ICU. As controls, we selected lung tissues of four patients died in 2017 and 2018 at Luigi Sacco Hospital: 2 ARDS cases (one H1N1 influenza virus pneumonia and one bacterial pneumonia), one case of Legionella pneumonia and one case of bacterial pneumonia without clinical ARDS. Tissues were fixed in 10% buffered formalin and embedded in paraffin. Three-micrometre paraffin sections were stained by haematoxylin–eosin for histological examination. Additional samples were fixed in 2.5% glutaraldehyde and prepared for ultrastructural analysis. Thin sections were stained with uranyl acetate/lead citrate and examined by EM-109 ZEISS and CCD-MegaView G2 (I-TEM imaging platform software).

In each case, 20 capillaries of the alveolar septa were examined. Criteria for the selection of the capillaries were as follows: fully visible lumen in the field of observation and preservation of the endothelial cell profile.

For each capillary, the opening of the junctional complex and the presence of structural junctional cytoskeleton traces in the open flaps were evaluated and quantified.

Statistical analysis

Descriptive statistics were used to characterize the cohort of patients, with denominators reflecting the number of patients treated either in ICU or in IMW. Categorical data were compared using Fisher's exact test or a chi-square test. Between-group comparison of continuous variables was accomplished via Mann–Whitney test or one-way analysis of variance, as appropriate. Correlation between non-normally distributed variables was assessed with Spearman's rho. Comparison of survival curves was analysed with the Mantel–Cox test. Values are shown as median and interquartile range or range when appropriate. Statistical significance was defined as $P < 0.05$. Data were analysed with GraphPad Prism version 8.4.1 and SPSS version 26.

Results

From 21 February to 15 April 2020, 174 hospitalized patients with COVID-19-related respiratory symptoms met the inclusion criteria. Of these, 92 were admitted to the IMW and 82 to ICU. All patients came from the area of a large epidemic outbreak located in Lombardy, Northern Italy.

Table 1 shows the characteristics of the studied population at admission, main treatments and outcomes.

None of the patients met the diagnostic criteria of the International Society on Thrombosis and Haemostasis for disseminated intravascular coagulation [17]. In the whole cohort, median serum albumin concentration was below and median D-dimer value was above the laboratory reference values, but ICU patients displayed more pronounced hypoalbuminaemia and higher D-dimer than IMW patients (Figure 1). The degree of respiratory involvement, expressed by the partial pressure of arterial oxygen (PaO_2)-to-the fraction of inspired oxygen (FiO_2) ratio, and the CXR score, was worse in ICU patients.

To assess whether patients with diverse degrees of respiratory impairment presented differences in serum albumin, we divided patients into three groups according to either PaO_2-to-FiO_2 ratio (>250, 250–150, <150) or CXR score (0–5, 6–11, 12–18). Lower albumin values were found in patients with more severe respiratory involvement (expressed as either lower PaO_2-to-FiO_2 ratio or higher CXR score) (Figure 2a). Median serum albumin was 31 (27–33) g L^{-1} in patients with PaO_2-to-FiO_2 ratio > 250, 24 (20.75–27.5) g L^{-1} in those with PaO_2-to-FiO_2 ratio between 150 and 250, and 20 (18–24) g L^{-1} g L^{-1} in those with PaO_2-to-FiO_2 ratio < 150. Serum albumin concentration was significantly lower in patients with chest X-ray showing more diffuse and pronounced pulmonary involvement ($P < 0.0001$) (Figure 2b): serum albumin was 31 (26.75–35.25) g L^{-1} with CXR score < 5, 27 (24–32) g L^{-1} with CXR score between 6 and 11, and 21 (18–24) g L^{-1} with CXR score ≥ 12. The hospital LOS and the in-hospital death rate were higher in ICU than in IMW patients (Table 1). After stratification of patients by the median of serum albumin concentration in the whole series (24 g L^{-1}), mortality at 30 days from admission was significantly different (53.2% vs. 21%, $P < 0.0001$) (Figure 3).

In the first 48 hours after admission, qualitative proteinuria measurements were available for 77 ICU patients and were undetectable in 73 (94.8%). Furthermore, quantitative 24-hour proteinuria was available for 12 ICU patients with a median (interquartile range) of 76 (28.5–165) mg.

One patient had a history of chronic enteropathy (Crohn's disease). No other patients had history or signs of protein-losing enteropathy or malnutrition.

Besides, in order to get more insights into such a cytokine storm, we measured the concentration of the pro-inflammatory cytokine (IL-8) and the anti-inflammatory cytokine (IL-10), and proteins in the BALF of 26 critically ill patients during their first week of ICU stay. Median BALF IL-8 was 886.4 (432.9–2648) pg mL^{-1}, IL-10 337.7 (230.3–375.2) pg mL^{-1} and proteins 378.9 (215.6–726.1) μg mL^{-1}. We found a positive correlation between protein concentration and the pro-inflammatory IL-8 ($\rho = 0.61$, $P = 0.001$), and an inverse correlation between protein concentration and the anti-inflammatory IL-10 ($\rho = -0.81$, $P < 0.0001$) in BALF (Figure 4).

Major histological findings were denudation of alveolar membranes and residual hyaline membrane deposition (Figure 5). There were proliferation and hyperplasia of type 2 pneumocytes in all the samples. The interstitium was focally expanded by mild fibrosis, oedema and moderate lymphocytic infiltrate.

Remarkably, the ultrastructural analysis of the autopsy lung tissues revealed the extensive opening of junctional complexes (JCs – tight, adherens and gap junctions) in eight out of ten patients and depletion of surfactant in type 2 pneumocytes of most cases (Figure 6a-e). Moreover, viral particles with morphology and intravacuolar localization coherent with coronavirus [18] were found in the cytoplasm of pneumocytes (Figure 6f) and endothelium (Figure 6g).

Evaluation of the junctional complexes through the above-mentioned quantitative approach revealed that endothelial damage was much more pronounced in COVID-19 as compared to H1N1- and bacterial-ARDS cases, and to Legionella and other bacterial pneumonia without ARDS (Table 2). Patients with COVID-19 had more capillaries with opening of the junctional complexes and open capillaries with the presence of structural junctional traces in the open flaps than the controls. Interestingly, ultrastructural findings in tissues of five patients with COVID-19 revealed the presence of multiple spots with cytoskeletal traces per capillary, a finding which was never identified in controls.

Table 1. *Characteristics of the studied population*

	ICU (n = 82)	IMW (n = 92)	P
Age, years	62.5 (48.7–68)	60 (49–73)	0.68
Male gender, n (%)	65 (79.3)	56 (60.9)	0.008
BMI	27.8 (25.4–31.9)	26.6 (23.9–29.8)	0.35
Comorbidities, n (%)			
Smokers[a]	11 (13.4)	47 (51.1)	<0.0001
Cardiovascular disease	39 (47.6)	41 (44.6)	0.76
Respiratory disease	3 (3.7)	12 (13)	0.03
Diabetes	11 (13.4)	26 (28.3)	0.025
Cancer	5 (6.1)	12 (13)	0.13
HIV	2 (2.4)	4 (4.3)	0.68
Immunosuppressive therapy	4 (4.9)	4 (4.3)	1
Symptoms to hospital admission, days	6 (4–9.2)	8 (6–11)	0.019
Hospital admission to CPAP, days	1 (0–3)	1 (0–2)	0.99
CPAP to mechanical ventilation, days	2 (1–4)	3 (1.5–4)	0.73
Fibrinogen, mg dL^{-1}	700 (700–700)	700 (600–700)	0.21
D-dimer, ng mL^{-1}	2374 (1186–7097)	953 (501–2349)	<0.0001
Haemoglobin, g dL^{-1}	12.7 (11.5–13.8)	12.9 (12.2–14.08)	0.93
Haematocrit, %	38 (34–41)	38.5 (36–41)	0.12
Platelets, 10^3cells mL^{-1}	230 (173–309.7)	205 (137.2–303.2)	0.11
WBC, cells mL^{-1}	8375 (6058–12013)	5885 (4500–9505)	<0.0001
Neutrophils, cells ml^{-1}	7526 (4922–10547)	3910 (2440–7185)	<0.0001
Lymphocytes, cells ml^{-1}	661 (460–935)	1062 (760–1513)	<0.0001
S-creatinine, mg dL^{-1}	0.9 (0.74–1.21)	0.88 (0.69–1.07)	0.37
LDH, U L^{-1}	537 (416–666)	303 (242–389)	<0.0001
S-albumin, g l^{-1}	20 (18–23)	28 (24–33)	<0.0001
AST, U L^{-1}	53 (36–76)	39 (28–61)	0.01
ALT, U L^{-1}	40 (22–72)	30 (21.75–61.25)	0.07
S-bilirubin, mg dL^{-1}	1.2 (1.2–1.2)	1.19 (1–1.2)	0.02
S-lactate, mmol L^{-1}	1.3 (1–1.5)	1.2 (1–1.5)	0.63
CRP, mg L^{-1}	176 (94–278)	70 (24–140)	<0.0001
Mechanical ventilation, n (%)	78 (95.1)	4 (4.3)	<0.0001
PEEP, cmH_2O	13.5 (12–16)	12 (10–12.5)	<0.0001
Respiratory rate, bpm	20 (18–24)	20 (18–26)	0.1
Tidal volume, mL	550 (500–600)		
Respiratory system compliance, mL cmH_2O^{-1}	38 (31–46)		
Arterial pH	7.35 (7.29–7.43)	7.46 (7.43–7.49)	<0.0001
Arterial pO_2, mmHg	83 (71–102)	89.5 (72.25–111)	0.27
Arterial pCO_2, mmHg	46 (40–52)	38 (34–41)	<0.0001
PaO_2-to-FiO_2 ratio	111 (91–151)	243 (148–376)	<0.0001
Chest X-ray score, n (%)			
0–5	1 (1.2)	47 (51.1)	<0.0001
6–11	5 (6.1)	25 (27.2)	0.0002

Table 1 (*Continued*)

	ICU (n = 82)	IMW (n = 92)	P
12–18	76 (92.7)	14 (15.2)	<0.0001
Missed	0	6 (6.5)	0.03
New RRT during hospital stay, n (%)	19 (23.2)	0	<0.0001
Tocilizumab, n (%)	25 (30.5)	18 (19.6)	0.11
Corticosteroids, n (%)	5 (6.1)	3 (3.3)	0.48
Remdesivir, n (%)	34 (41.5)	0	<0.0001
Hydroxychloroquine, n (%)	60 (73.2)	79 (85.9)	0.057
Lopinavir/ritonavir, n (%)	53 (64.6)	43 (46.7)	0.22
ICU outcome			
Mechanical ventilation length, days	11 (5.2–16.7)		
LOS, days	14 (8.7–18.2)		
Dead, n (%)	41 (50)		
Hospital outcome			
LOS, days	25 (16–42.2)	8 (5–14)	<0.0001
Dead, n (%)	44 (53.6)	20 (21.7)	<0.0001

Data are shown as median (25^{th}–75^{th} percentiles) or as n (%) when indicated. ICU, intensive care unit; IMW, intermediate medical ward; BMI, body mass index; CPAP, continuous positive airway pressure; PEEP, positive end-expiratory pressure; CRP, C-reactive protein; WBC, white blood cells; CXR, chest X-ray Brixia score; RRT, renal replacement therapy; LOS, length of stay.
[a]Present or past smoking.

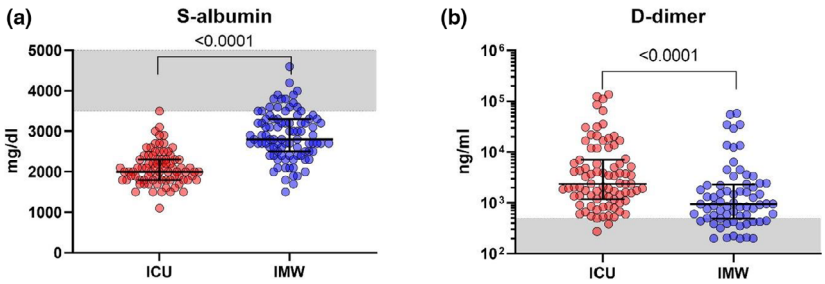


Fig. 1 *Dot plots of serum albumin (panel a) and D-dimer (panel b) at admission. Grey bands represent reference ranges for healthy populations. Most of patients had out-of-range values.*

Discussion

The main finding of our study is that the hypoalbuminaemia frequently found in patients with COVID-19 is linked to the degree of respiratory impairment, with lower serum levels in those with a lower PaO_2-to-FiO_2 ratio or a higher CXR score. In addition, patients with serum albumin levels < 24 g L^{-1} displayed a higher in-hospital mortality. Finally, patients admitted to ICU presented with worse hypoalbuminaemia as compared to those admitted to the IMW, and this was accompanied by worse respiratory impairment and a higher mortality rate.

Hypoalbuminaemia is often associated with inflammatory conditions, and it is a common feature of all acutely ill patients. In this scenario, it is linked to both increased vascular permeability (with augmented distribution volume of albumin) and shortened albumin half-life (altered kinetics with neonatal Fc receptor downregulation and

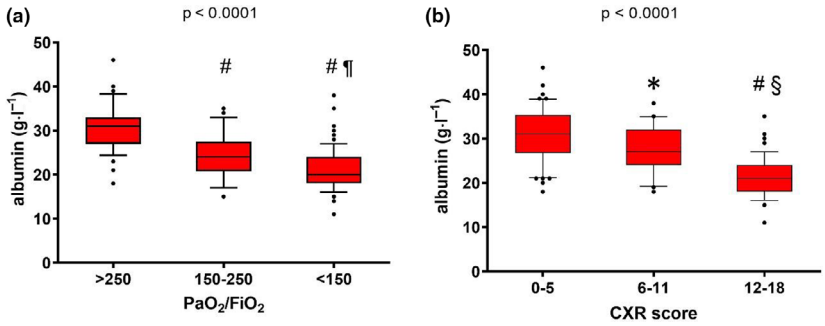
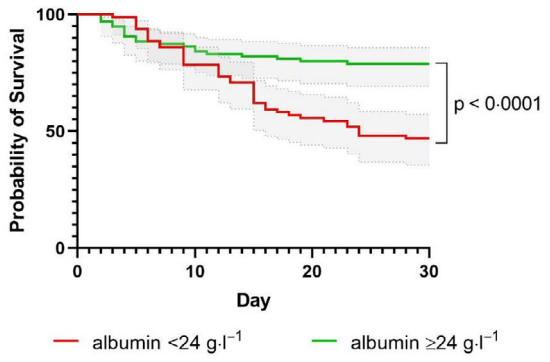


Fig. 2 *(a) Serum albumin concentration and PaO_2-to-FiO_2 ratio (PaO_2/FiO_2): serum albumin values were lower in patients with lower PaO_2/FiO_2, index of worsening respiratory function (P < 0.001). # P < 0.001 vs. PaO_2 to FiO_2 > 250; ¶ P < 0.05 vs. PaO_2 to FiO_2 150–250. (b) Serum albumin concentration and chest X-ray (CXR) score (P < 0.001): higher CXR scores, indexes of more diffuse and clear pulmonary involvement, are found in patients with lower serum albumin concentrations. *P < 0.05 vs. 0–5 CXR score; # P < 0.001 vs. 6–11 CXR score; § P < 0.001 vs. 0–5 CXR score.*



Day	0	5	10	15	20	25	30
albumin <24 g·l^{-1} (n)	79	78	68	56	45	41	37
albumin ≥24 g·l^{-1} (n)	95	86	82	79	77	76	75

Fig. 3 *First 30-day survival curves in the whole cohort according to serum albumin concentration at admission. Bands represent 95%CI.*

increased intracellular breakdown) leading to decreased total albumin mass, despite increased fractional synthesis [19]. In hyperinflammatory states, such as trauma, shock or infection, hypoalbuminaemia acts as a negative prognostic marker, and hypoalbuminaemic patients are more prone to react inadequately to a so-called second hit, such as surgery. Decreased albumin levels are linked to poor outcomes and reduced life expectancy [20]. Nonetheless, no clear benefit has been demonstrated for albumin solution administration in critically ill patients, although a positive effect has been hypothesized in patients with septic shock [21].

A major pathophysiological mechanism of hypoxaemia in the acute phase of ARDS due to viral pneumonia (i.e. influenza A) is the direct viral injury to the epithelial–endothelial barrier, resulting in proteinaceous oedema. Usually, the main resistance to protein flux across the epithelial–endothelial barrier is the alveolar epithelium and, to a lesser extent, the endothelial layer, which both depend on the integrity of their JCs [22, 23]. However, endothelial cells are the predominant cell line in the lung, accounting for 30% of all cell types [24]; thus, they exert a pivotal role in the regulation of transmembrane flux. Autopsy findings of patients included in this study showed widespread damage of both epithelial and endothelial alveolar cells resulting in a combined injury of both sides of the alveolar–capillary interface. This unfavourable combination allows the passage of fluids and proteins from the intravascular to the alveolar spaces. Moreover, endothelial injury may act as a procoagulant trigger [25], and in association with capillary fluid, depletion may ultimately favour intravascular microthrombosis. Taken together, these results support the hypothesis that, in COVID-19, capillary leak syndrome might significantly contribute to the development of hypoalbuminaemia, which in turn could be interpreted as a marker of disease severity, which has been described in most autopsy series [8, 26].

The term 'capillary leak syndrome' refers to leakage of intravascular fluids into the extravascular

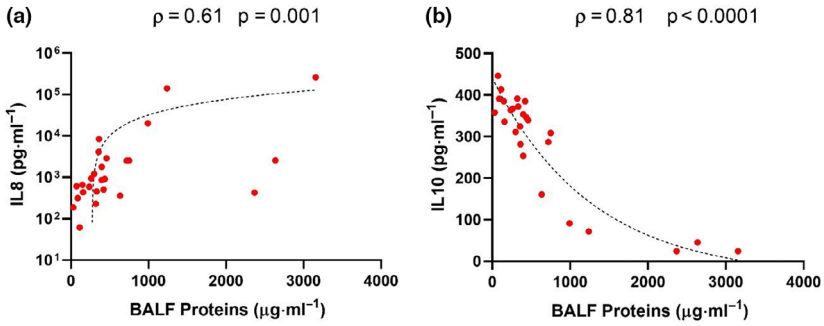


Fig. 4 *(a) Correlation between concentrations of pro-inflammatory interleukin 8 (IL-8) and proteins in the bronchoalveolar lavage fluid. (b) Correlation between concentrations of anti-inflammatory interleukin 10 (IL-10) and proteins in the bronchoalveolar lavage fluid (BALF).*

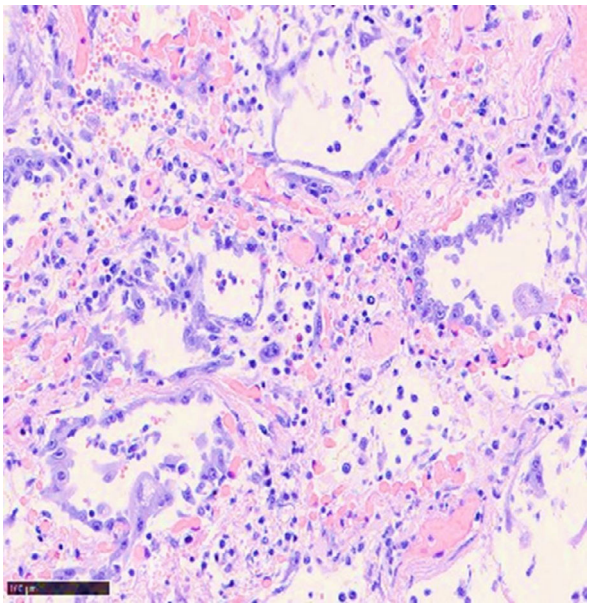


Fig. 5 *Histological picture of lung parenchyma with hyperplastic and atypical type II pneumocytes. Septa are wide for fibrosis ad accumulation of inflammatory cells (haematoxylin–eosin, OM ×20).*

space. The condition may stem from a variety of underlying clinical conditions and pathophysiological mechanisms. In a minority of cases, capillary leakage is considered to be the *primum movens* of the clinical condition, with no identifiable endothelial injury and has transient features as in the idiopathic systemic capillary leak syndrome (ISCLS), belonging to the group of Paroxysmal Permeability Disorders (PPDs) [27]. Either primary or secondary capillary leak syndrome might be associated with a variety of clinical pictures, ranging from asymptomatic states to generalized oedema and life-threatening multiple organ failure, requiring urgent intervention.

In our COVID-19 cohort, electron microscopy imaging confirms the opening of interendothelial junctions, in association with an inflammatory state, as corroborated by cytokine findings into the BALFs. These findings are of pivotal importance since it is known that, whilst fluids and solutes may cross the endothelial barrier via diffusion and filtration, the passage of macromolecules and proteins from the intravascular to the interstitial space is tightly controlled by interendothelial junctions (paracellular pathway) or biochemical-vesicular transport systems (transcellular pathway) [28]. Furthermore, the presence of virions within the cytoplasm of both type 2 pneumocytes and lung capillary endothelial cells may suggest also a direct viral damage to the alveolar–capillary barrier.

High protein concentrations in BALF (with no evidence of proteinuria or protein-losing enteropathy) further support our hypothesis, confirming passage of proteins probably through open JCs.

Some major differences between the suggested pathological mechanism in COVID-19 and what is known about PPDs need to be elucidated. The most important one is related to the site-specificity of COVID-19 endothelial hyperpermeability: the pulmonary vascular bed (as well as cerebral vessels) is usually spared in PPDs, whilst it is the key player in COVID-19. This might be at least partially due to SARS-CoV-2 tropism, which seems to be closely

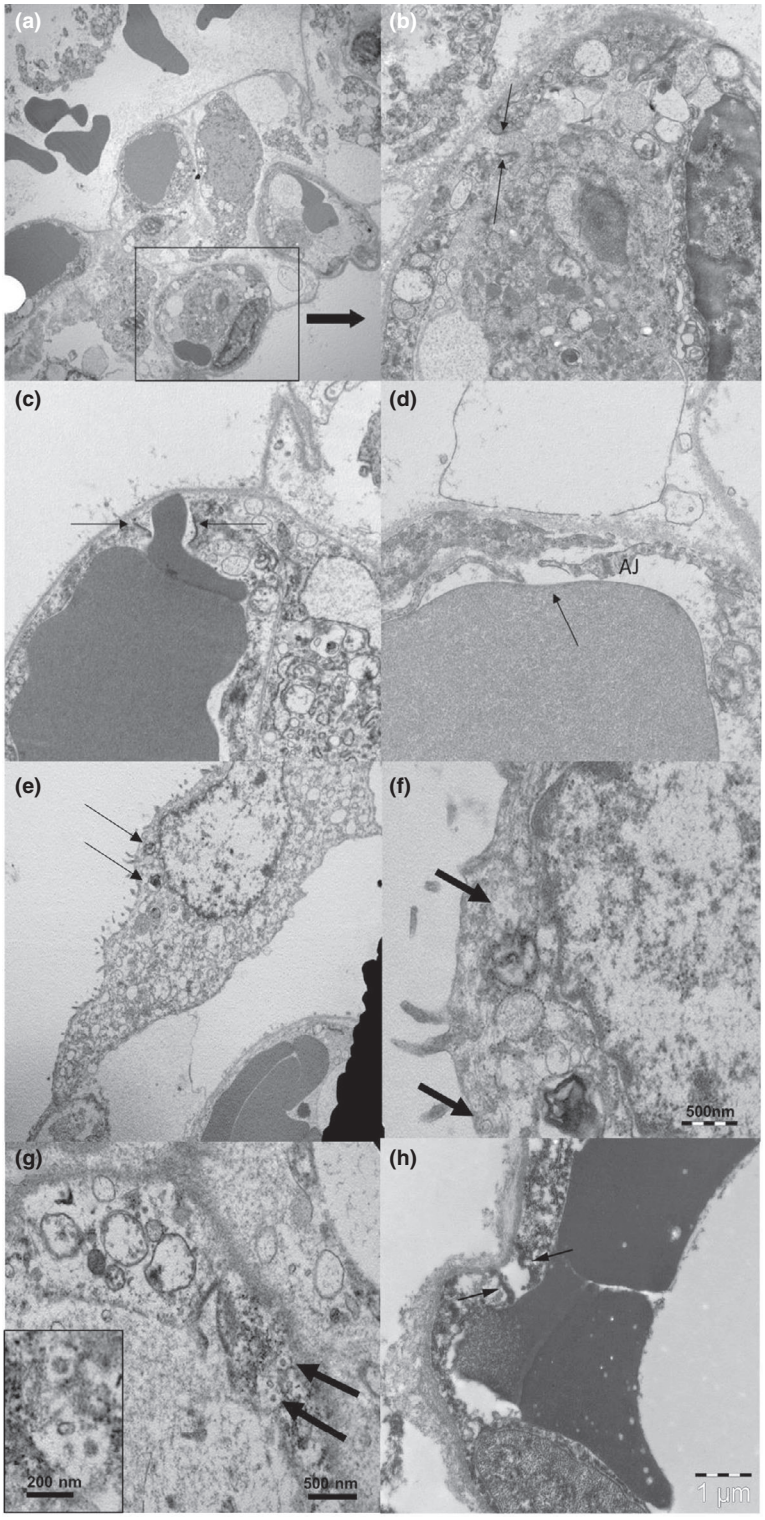


Fig. 6 *Panels a-g: Ultrastructural images of lung septa between two disepithelized alveoli (patients with COVID-19). One of the capillaries (a) shows an evident gap which at higher magnification (b) appears caused by the opening of the junctions. In panels b and c, the opened junctional complexes still show traces of the cytoskeletal structures (actin) of the adherens junctions that appear as an electrondense plasmalemmal thickening. The passage of red cells (c) can be observed through the open junction. In panel d, there is a small adherent junction still preserved (AJ) whilst the flaps of the capillary (arrow) are completely separated without a trace of residual junctional structures. Panel e: complete detachment of a type 2 pneumocyte from the alveolar basal membrane. Residual small aggregates of surfactant (arrows) are found. In panels f and g: virions (arrows) in cytoplasmic vacuoles of a pneumocyte (f) and an endothelial cell (g). Virions had an average diameter of 82 nm, and viral projection about 13nm in length (inset left up, OMx85000). Panel h: Lung septal capillary of H1N1 patient. Ultrastructural image of endothelial gap with traces of junctional structures (arrows). (OM: panel a ×3000; panels b-c-d-h ×12000; panel e ×4400; panel f and g ×20000; inset panel g ×50000).*

Table 2. *Ultrastructural capillary characteristics of COVID-19 patients and controls*

	Capillaries with opening of the junctional complex	Open capillaries with the presence of structural junctional traces in the open flaps
COVID-19 1	9	3
COVID-19 2	12	7[a]
COVID-19 3	10	3
COVID-19 4	4	4[a]
COVID-19 5	6	5
COVID-19 6	8	7[a]
COVID-19 7	0	0
COVID-19 8	6	3[a]
COVID-19 9	15	6[a]
COVID-19 10	0	0
Bacterial pneumonia-ARDS	1	0
H1N1 pneumonia-ARDS	4	2
Bacterial pneumonia	1	0
Legionella pn. pneumonia	1	1

[a]Presence of multiple spots with cytoskeletal traces per capillary.

related to the expression of specific receptors (as ACE2 receptors) on pneumocytes. Nonetheless, the vascular endothelium should be regarded as a complex organ whose phenotypic heterogeneity could also partially explain variable behaviour across different sites [29, 30].

Moreover, whilst ISCLS is considered to be a primarily functional condition, with no detectable damage to endothelial cells (despite reports of elevated levels of pro-inflammatory mediators and neutrophil granule components in acute ISCLS sera) [31], in COVID-19 a process of programmed cell death and endothelial injury is also involved [13].

In addition, endothelial injury in patients with COVID-19 may underlie diffuse intravascular coagulation of lung microvessels (<1 mm in diameter) [26]. Endothelial injury and JC loosening expose plasma proteins to tissue factor and to the extracellular matrix, which leads to intra-alveolar activation of coagulation and thrombin generation [25], with consequent intravascular coagulation in small vessels, whilst larger vessels are mainly spared [26].

The comparison between postmortem findings in COVID-19 and other diseases known to affect the pulmonary vascular bed (as H1N1- and bacterial-ARDS as well as Legionella and other bacterial pneumonia) adds further robustness to our results. In fact, it highlights that endothelial damage, even though it is not specific of COVID-19, occurs at a much higher degree in COVID-19, thus supporting the hypothesis that opening of interendothelial junctions is far from being an epiphenomenon of the disease and may actively contribute to the progression and worsening of the condition. Furthermore, the findings of spared junctional complexes in two mechanically ventilated patients with COVID-19 of our series may indicate that the underlying pathophysiological mechanism is not simply due to the repeated stretching caused by mechanical ventilation.

Our observations pave the way to a more responsible management of patients with COVID-19, avoiding useless (and potentially harmful) administration of albumin (which is likely to extravasate due to loosening interendothelial junctions) and acting on the underlying pathological mechanism. A phase IIb randomized, placebo-controlled study (EUDRACT No. 2017-003855-47) is already ongoing to investigate the safety and preliminary efficacy of sequential multiple ascending doses of solnatide, a 17 residue peptide mimicking the lectin-like domain of TNF [32], to treat pulmonary permeability oedema in patients with moderate-to-severe ARDS in Germany and Austria.

Other molecules are under investigation for their ability to stabilize the endothelial barrier. Amongst those is FX06, a naturally occurring peptide, Bβ15-42, derived from the E1 fragment of fibrin, which binds to VE-cadherin, preventing VE-cadherin-dependent transmigration of leucocytes and stress-induced rearrangement of the endothelial cell actin cytoskeleton leading to rupture of adherens junctions. FX06 has been used with promising results in a small cohort of sickest patient with

COVID-19, almost all needing extracorporeal membrane oxygenation [33].

To our knowledge, this is the first study which, through a multidisciplinary approach, investigates the causes underlying hypoalbuminaemia in COVID-19 and addresses its relationship with respiratory impairment. In addition, our findings suggest some similarities between COVID-19 and PPDs, which, if confirmed, could lead to useful clues for future treatment research. However, we are aware that our study has also some limitations. First, it is retrospective, and our findings will need confirmation in prospective studies. Moreover, we propose a pathophysiological model which necessarily needs validation.

In conclusion, hypoalbuminaemia is a frequent finding in patients with COVID-19 appears linked to the severity of lung injury. It might depend on the complex interplay between direct viral effects and the hyperinflammatory host reaction, which lead to endothelial dysfunction and pulmonary capillary leak syndrome. Further research is needed to get deeper insights into the mechanisms leading to this condition, and to assess whether endothelial dysfunction can be considered a new promising therapeutic target.

Conflict of interest

The authors have no conflicts of interest to declare.

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Authors' contribution

Maddalena Wu: Conceptualization (lead); Data curation (lead); Investigation (equal); Methodology (equal); Writing – original draft (lead). **Tommaso Fossali:** Investigation (equal); Writing – review and editing (equal). **Laura Pandolfi:** Investigation (equal); Writing – review and editing (equal). **Luca Carsana:** Investigation (equal); Writing – review and editing (equal). **Davide Ottolina:** Data curation (equal); Writing – review and editing (equal). **Vanessa Frangipane:** Investigation (equal); Writing – review and editing (equal). **Roberto Rech:** Investigation (equal); Writing – review and editing (equal). **Antonella Tosoni:** Formal analysis (equal); Writing – review and editing (equal). **Gianluca Lopez:** Investigation (equal). **Andrea Agarossi:** Investigation (equal); Writing – review and editing (equal). **Chiara Cogliati:** Investigation (equal); Writing – review and editing (equal). **Federica Meloni:** Data curation (equal); Supervision (equal); Validation (equal). **Beatrice Marchini:** Data curation (equal); Writing – review and editing (equal). **Manuela Nebuloni:** Data curation (equal); Formal analysis (equal); Investigation (equal); Supervision (equal); Validation (equal); Writing – review and editing (equal). **Emanuele Catena:** Supervision (equal); Validation (equal); Writing – review and editing (equal). **Riccardo Colombo:** Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (equal); Methodology (equal); Supervision (lead); Validation (lead); Writing – original draft (equal).

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