 doi:10.20944/preprints202204.0118.v1

SARS-CoV-2-Infection (COVID-19): Clinical Course and CAUSE (S) OF DEATH

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key words: SARS-CoV-2-infection,dehydration,hypoalbuminemia,pulmonary hypoxia,hyaline memrane,pulmonary engorgement,lung weight ,acute respiratory distress syndrome,(),diffuse alveolar damage (DAD)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 April 2022 doi:10.20944/preprints202204.0118.v1

ABSTRACT

Two years after first patients approached the emergency rooms of hospitals in Wuhan becouse of respiratory distress,thousend of SARS-CoV-2 infected persons continue to die every day worldwide.SARS-CoV-2-infected patients undergo a process of dehydration and malnutrition before they develop respiratory problems and approach the emergency room of a hospital.This is,in many cases, the consequence of high fever which causes massive loss of fluids. In addition loss of appetite, is responsible for the deficit of protein intake.Most of the virus-infected patients admitted to the emergency room are therefore hypovolemic and hypoproteinemic and suffer of respiratory distress accompanied by ground grass opacities at CT-scan of the lungs.Critically ill patients are treated following the guidelines for treatment of septic shock but with „conservative“ fluid replacement and administration of diuretics to assure sufficient hourly urine production.

The combination of conservative fluid administration with reduced protein content in the enterally administered diet, together with administration of diuretics, has severe hemodynamic consequenses in mostly aged,dehydrated,critically ill patients. Many of them will develop acute kidney injury in the next 24 hours.

In most of the cases, patients continue to loose weight by loosing skeletal muscle mass.

Ischemic damage in the lung capillaries is responsible for the acute respiratory distreass syndrome (ARDS) and for the hallmark of autoptic findings,diffuse alveolar damage (DAD) characterized by hyaline membrane formation,fluid invasion of the alveoli recruitment of some inflammatory cells and progressive arrest of blood flow in the pulmonary vessels.The consequence is progressive congestion , increase of lung weight and progressive hypoxia (progressive severity of ARDS).Sequestration of blood in the lungs worsen hypovolemia and ischemia in different organs.This is most probably responsible for recruitment of inflammatory cells and for persistance of elevated serum levels of positive acute-phase markers and of hypoalbuminemia.

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Autoptic studies have been performed mostly in patients who died in the ICU after SARS-CoV-2-infection because of progessive ARDS.In those patients, tubulus epithelium necrosis in the kidney is a frequent finding as it has been the case in the first SARS-CoV-1 pandemic.In the death certification charts, many times weeks after first symptoms have started ,cardiac arrest is the cause of death after respitatory insufficiency.

Replacement therapy with sufficient amount of fluid and albumin should be part of the early individualized life-saving supportive measures avoiding mechanical ventilation.

Key words: COVID-19,autopsy, lung, bronchial epithelium,nasal mucosa,albumin infusion,tissue ischemia,dahydration, liquid and nutrition,cardiac arrest,cause of death.

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Ramadori Giuliano MD

More than two years after the beginning of the pandemic, more than 470 million people have contracted the HCoV-2 infection and more than 6 million people have died(1).The true number of death could be however much higher (2,3)

While the age of the patients hospitalized in Wuhan was mostly under 65 years, (4-8) the first children of a mean of 3 years of age were observed from january 7 to january 15,2020(9) with an ICU admission. Nine more cases observed in january/february 2020 were described (10). Children continue to be infected and eventually die also in the western countries (11,12).

As in most of non- hospitalized cases but also in hospitalized persons who die of HuCoV-2 infection, the cause of death is respiratory insufficiency (ARDS) with the classical CT-scan finding of the chest of the so called ground glass opacities (GGO), supposed to be due to directly induced viral damage, it is important to reconstruct the chronologic sequence of clinical events and of the changes detected by radiological imaging and to correlate them with the available autoptic findings published until recently.

Most of the reports are based on data obtained on hospitalisation whereas data collected at the beginning of the symptoms and during the prehospitalisation time are scarse.

Treatment guidelines therefore mostly concern the antiviral treatment and the suppression of the acute-phase reaction with experimental drugs, starting at the beginning of the hospitalization before entering and/or during the ICU-stay (13-15) which have been previously studied in therapeutic trials under different protection conditions for the health care professionals .

Compared to the high number of therapeutic trials performed on hospitalized patients , much less attention has been paid to the treatment of the initial symptoms in order to prevent physical deterioration and the need of hospitalisation(16-22).

The clinical course of HuCoV-2- and of Influenza-infection is quite similar(23-28):runny or stuffy nose,headace,dry cough sore throat,fatigue,muscle pain fever of different height and length,inappetence,vomiting , diarrhea which may then lead to developement of shortness of breath or difficulty of breathing.To these symptoms can be added oliguria and loss of body weight (29,30).

In many cases,because of the fear of infection transmission within the family member as soon as the oximeter shows a p02<93%, help in the emergency room of an hospital is sought even without symptoms of shortness of breath or increase of breath frequence.

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At this time,several days after beginn of symptoms, chest x-ray studies may, at first, result normal and CT-values of PCR-analysis may suggest low viral load.

Chest-CT-scan performed at the same time or one or two days later may show ground glass opacities in the subpleural dorsal and in basal portions of the lungs (Fig.1) suggesting a risk of progress into acute respiratory distress syndrome (ARDS).

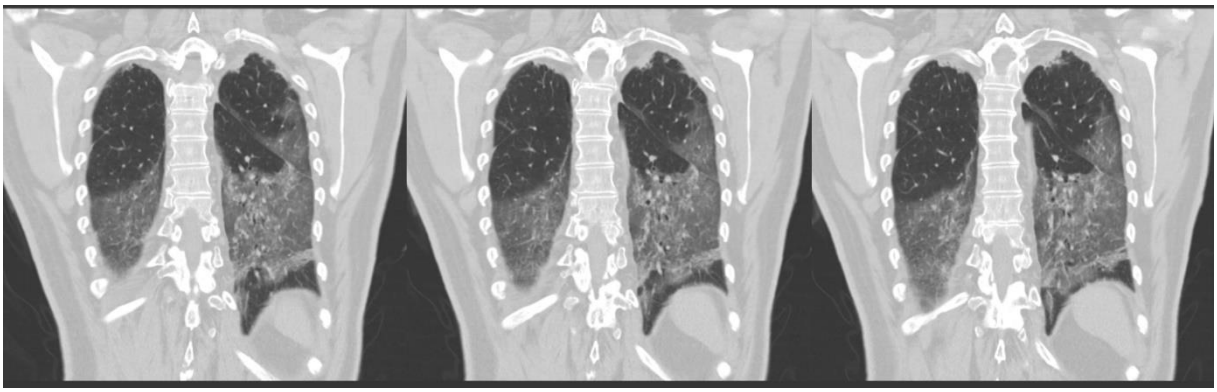

Fig.1: *Two days before admission the conventional radiology of the chest was normal and the patient was released from the emergency room.He was readmitted two days later. CT-scan of the chest in the 74 year-old COVID-19-patient showed bilateral opacities.At time of this CT-scan CRP serum level was normal.Patient was then mechanically ventilated but could be released after 8 weeks of stay at the ICU and send for rehabilitative therapy.*

A further decrease of Pa02 may lead to the decision of mechanical ventilation (31,32) with the diagnosis of ARDS often depending on setting and circumstances (33) which may also have been influenced,at least in part by the administering of experimental drugs.

The first person who officially died of COVID-19 in Italy in Vo`Euganeo on february 20th was a 78 year-old man (34). Thereafter most of the patients who died of COVID-19 in Italy were over 80 years old(35-37). The same events were observed in Germany, in Denmark,Sweden and in the other countries of the western world (38-42) where more than two thirds of the people who died of COVID-19 where old or very old , although the majority of the infected people ranged between 15-60 years of age .

This discrepancy has been explained by the special frailty of the older patients.

persons due to the presence of different comorbidities like hypertension, diabetes, chronic lung or kidney disease (43-46). Most of the old persons who were infected and died lived in assisted retirement facilities.Mortality in such houses however was dependent on the care organisation.In fact in some of them mortality could be strongly reduced or even completely avoided (47).

Although several clinical patterns have been reported, fever and dry cough are the most frequent symptoms reported as the beginning of the disease (20% of the PCR-positive persons) with further (6-9 day after beginn of first symptoms) worsening characterized by dyspnea of different degrees (5%) leading to hospitalisation (48,49) and eventually to ICU (50).

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Anamnestic informations given by the patients demselves or by their relatives concerning the beginning of the symptoms,the clinical developement and the duration of the symptoms in the pre-hospitalisation time have been often unprecise.

In fact, as many old patients were brought to the hospitals from assisted care facilities it was very difficult to ask about height and duration of fever,fluid and calory uptake , changes in body weight since symptoms first appeared and which drugs were regularly administered.As the modern doctor has less and less time for contact with his patients and with their families (51,52),this may have been even more difficult during the first wave of the SARS-CoV-2-pandemic,because of the exceptional situation in the emergency room due to overcrowding and the fear of contagion by the diffuse lack of preventive measures among the health care personnel.

The physical conditions of the old infected patients,which normally require a careful examination of the skin and mucosal surfaces, hydration status examination has often been impossible and therefore not reported (51,52).

In many cases, immediately after admission, patients have been sent to the radiology department for diagnostic chest-CT-scan (53).

This, In fact, was due,at least in part, to the overcrowding of the emergency rooms, because of lack of time to wait for the virological diagnostic procedure (where available).It also led to further delay of supportive therapy (administration of fluids and nutrition (54-56**).**

Diagnostic pictures from this source have then dominated the further clinical proceedings,e.g.hospitalisation and eventually mechanical ventilation.

Administration of antiviral drugs and even more frequently of corticosteroids was considered a priority (8,43,54,55) as it was suggested that these would be effective if administered early(56).

Two publications (7,57) concentrated on the changes of serum levels of inflammatory cytokines,mainly Interleukin-6,TNF-alpha,Interleukin-1-beta and also interleukin-8 (58) during the hospitalisation.In the first publication a decrease of the acute-phase cytokines IL-6 and TNF-alpha was observed in the serum of patients who were discharged from the hospital.IL-1-Beta serum level was slightly elevated only in three of 27 patients studied (**7**).In the second publication (57) serum level of IL-6,TNF-alpha and IL-8 were significantly elevated in COVID-19-patients,IL-6 being the most prominent cytokine (all in the order of magnitude of picogram/ml).Il-1-beta-serum level was always low or at the limit of detection.

Therefore a difference in the behavior of the serum levels of these acute-phase cytokines was observed and „different cutoffs were chosen for further statistical analysis“ (57).Authors found that the serum level of the cytokines was differently associated with comorbidities.

The first determination of IL-6 and TNF-alpha-serum levels after hospitalisation was indicative for the prognosis of the patient,higher levels having a bad prognostic meaning.Especially IL-6- and IL-8-initial serum levels were closely correlated with severe disease with signs of organ failure showing disastrous lung imaging,reduction of creatinin clearance,the need for vasopressors and use of ventilation.

Under the effect of experimental drugs such as IL-6-receptor antagonists,corticosteroids or remdesivir there was a fluctuation of IL-6 serum levels with a potentially positive effect on prognosis. Changes of viral load were not reported.

The discussion about the source of the acute-phase cytokines and of their role and meaning within the clinical picture of COVID-19 focused more on the possible use of the measurement of serum levels to determine prognosis.

While the main acute-phase cytokine, Il-6 is mainly synthesized by macrophages recruited at the site of „aggression“ and tissue damage,the chemokine IL-8 is also locally synthesized by every cell under „attack“ and contributes to the local recruitment of inflammatory cells.

Increase of IL-8-serum levels under such acute-phase conditions, however, is more dependent on the magnitude of Il-6-serum level,which induces production of the chemokine in the liver (59) which has to be high enough to induce increased release of granulocytes from the bone marrow (60,61) .

In fact, especially in viral diseases,like COVID-19 the serum level of IL-6 and consequently of IL-8 does not seem to be high enough to induce the massive increase of leukocyte count which is characteristic for bacterial infections or for other acute clinical situations (62-65).

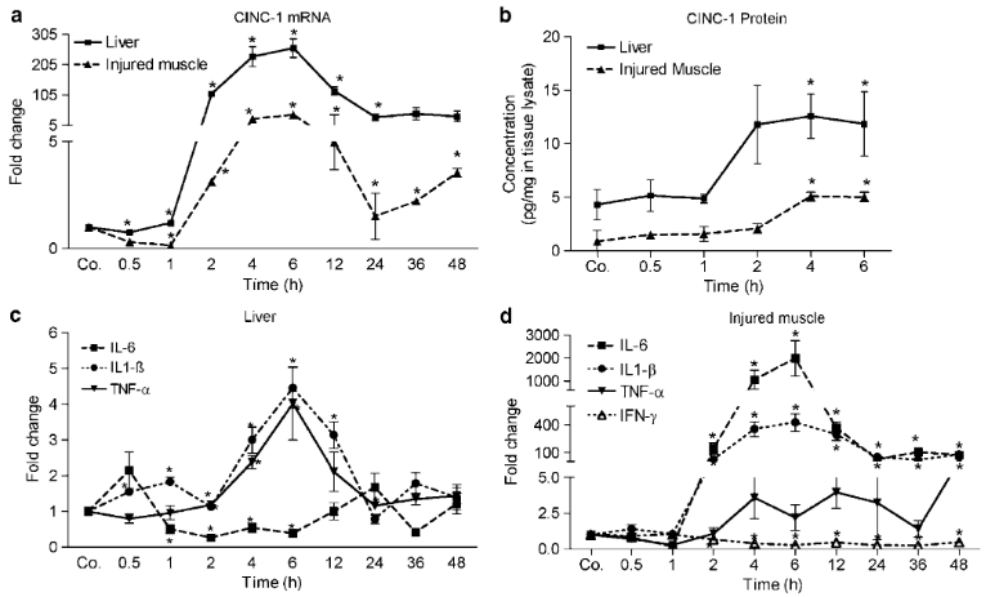

Figure 7 Expression of CINC-1 mRNA **(a)** and protein **(b)** in liver and injured muscle, and mRNA of acute-phase cytokines in liver **(c)** and injured muscle **(d)** at different time points after intramuscular TO treatment. The mRNA expression was quantified by real-time RT-PCR **(a**, **c** and **d)**, CINC-1 protein was quantified by ELISA in lysates of liver and injured muscle **(b)**. The CINC-1 concentrations were related to the total protein of the lysates, based on total protein concentration measurements **(b)**. CINC-1 protein was induced in liver 2 h after TO injection, while at the site of TO injection the induction occurred only 4 h after the TO injection. Results represent mean value±s.e.m. of three animals. $*P<0.05$.

Laboratory Investigation (2006) 86, 800–814

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Fig.2: *Induction of chemokine- (CINC-1, Il-8)-gene-expression(mRNA and protein) in the liver and in the muscle of TO-treated rats (a,b).Cytokine-gene-expression in the liver and in the injured muscle at different time after intramuscular TO-administration (c-d).The enormous increase oft he IL-6-gene-expression in the injured muscle parallels the 300 fold- increase of Il-8-gene-expression in the liver while the increase of expression the same gene was temporarely about five fold (a)(58).*

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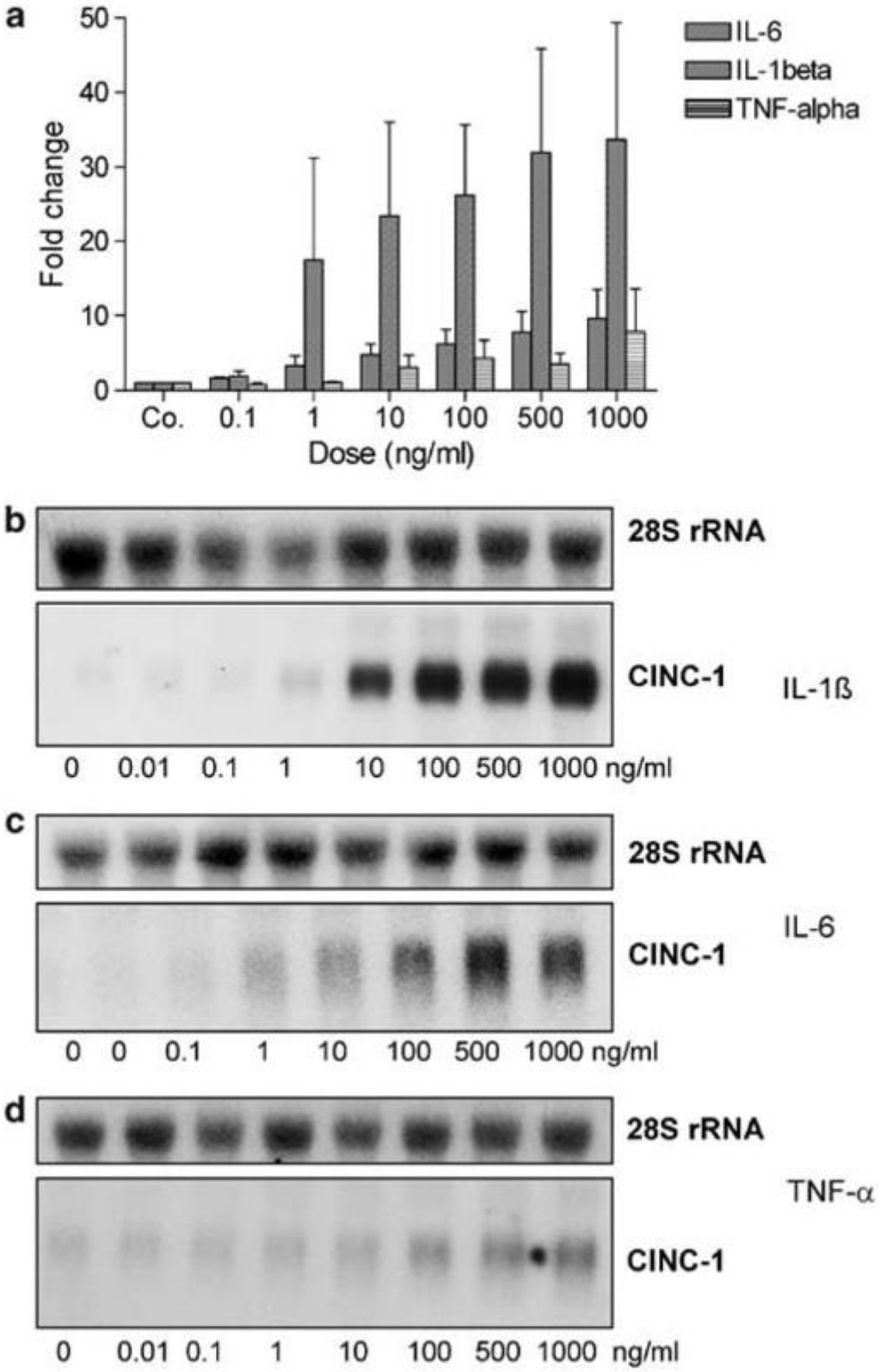

Fig.3: *Induction of chemokine- (CINC-1, Il-8) -gene-expression in cultured rat hepatocytes by treatment with different amounts of Il-1-beta, Il-6 or TNF-alpha.Northern blot analysis of total RNA hybridized with the specific radioctive cDNA.The quantification of the signal in*

relation to that of the house-keeping- RNA indicates that IL-6 (but also Il-1) has the highest potency of induction of IL-8 in hepatocytes(58).With permission.

A great amount of data has been generated mostly by analyzing samples from hospitalized patients (66-84) but in most of the cases,with a few exceptions, the effect of the different drugs administered immediately on admission (7,55) has not been considered.

Interestingly a previously published work from France (75) seems to confirm the laboratory changes which could be supposed from the publication of Xie (26) and coworkers.

The French authors(75) followed the laboratory changes observed in 162 patients during their hospital stay.They showed an increase of the number of patients developing acute kidney injury (AKI) from 0% on admission to 21.4% at day 14.At the same time increased osmolality and increased CRP- serum levels were observed while albumin serum level (below 25 g/L) continuosly decreased.

Hyperamylasemia was also present but morphological signs of acute pancreatitits were lacking .

However,although the data strongly suggests hypovolemic pre-renal insufficiency as well explained by Sise et al (80) and by Bowe et al(81),no information has been given about the serial measurements of blood volume .

On the contrary , viral replication in organs outside the respiratory tract has been repeatedly made responsible for organ „injury".Continuous determination of the viral presence during hospitalisation has however been rarely reported.

The work published by Xie et al.(26) shows interesting aspects which derive not only from the description of the changes obseved during hospitalisation in COVID-19 patients but through the comparison with patients admitted to the hospitals because of influenza infection in the 3 years preceeding the COVID-19 pandemic.Although the admission characteristics of the influenza infected (75% white and 25%black) and of those COVID-19-patients (50% white and 50% black) were quite similar, in white patients with COVID-19, mortality,although lower than in the black population, was more than three times higher than in the influenza-infected patients.This because of the worsening of the clinical conditions occurring after hospitalisation (26).

In an attempt to create the basis for personalized treatment(s), mortality risk scores have been developed, by different means retrospectively, analyzing the clinical data and laboratory parameters derived from blood and sometimes urine samples taken on admission to hospital, mostly under the conditions mentioned above **(**85-92).

The laboratory data collected also during the stay in hospital, which were used to extrapolate the clinical developement and somehow to explain clinical processes which then ended,in part of the patients with death,measures such as transfer to ICU,use of mechanical ventilation and kidney replacement therapy were used as prognostic markers but therapeutic consequences were not discussed.

The negative prognostic value of hypoalbuminemia was repeatedly described (93) at the beginning of the pandemic.The crucial importance of normal serum albumin level has been, however, underestimated (94-98)

The increased use of diuretics, which are routinely given on the assumption that ARDS is a kind of non-cardial„edema“ of the lung, has only been reported in a recent publication (99).

Instead,use of vasopressors is quite often mentioned also in guidelines without explanation for their being necessary, even when cardial arrythmias can occur as a consequence without signs of cardial damage or insufficiency (100).

Hypoxia without a cardial cause represents *the conditio sine qua non* for the diagnosis of acute respiratory distress syndrome (ARDS) when hypoxemia occurs in COVID-19-patients showing diffuse ground-glass opacities at the CT-scan of the chest.

Compared to patients with influenza infection,patients who were diagnosed COVID-19 were found to have an increased risk of extrapulmonary organ failure associated with an increased use of „health resources“.

The main question is therefore whether this developement after hospitalisation is due to viral replication or to the increased administration of drugs, which seem to aggravate in many cases preexsisting conditions such as hypoalbuminemia (86,93) and dehydration (29). These are eventually the basis for hemodynamic changes leading to most of the complications with the need of routinely administered catecholamins /(vasopressors) and to death especially in older persons.

This, although Huang J et al. (93) in their retrospective analysis of the charts of 299 COVID-19 patients found that hypoalbuminemia could predict outcome independently of age and comorbidities.

As in most of non- hospitalized cases but also in hospitalized persons who die of HuCoV-2 infection, the cause of death is respiratory insufficiency (ARDS) supposed to be due to directly induced viral damage, it is important to reconstruct the chronologic sequence of clinical events and of the changes detected by radiological imaging and to correlate them with the available autoptic findings published until recently.

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As mentioned above,the clinical course of HuCoV-2- and of Influenza-infection is quite similar(13,16,17,19,25,98,):runny or stuffy nose,headace,dry cough sore throat,fatigue,muscle pain fever of different height and length,inappetence,vomiting , diarrhea which may then lead to developement of shortness of breath or difficulty of breathing.To these symptoms can be added oliguria and loss of body weight (16,27).

In many cases,because of the fear of infection transmission within the family member as soon as the oximeter shows a p02<93%, help in the emergency room of an hospital is sought even without symptoms of shortness of breath or increase of breath frequence.

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Chest-CT-scan performed at the same time or one or two days later may show ground glass opacities in the subpleural dorsal and in basal portions of the lungs (Fig.1) suggesting a risk of progress into ARDS.

A further decrease of Pa02 may lead to the decision of mechanical ventilation (13,14) with the diagnosis of ARDS often depending on setting and circumstances (15) which may also have been influenced,at least in part by the administering of experimental drugs.

Acute Respiratory Distress Syndrome (ARDS):definition and possible causes

The definition of Acute Respiratory Distress Syndrome (ARDS) in adults was first described in 12 patients aged from 11 to 48 years by Ashbaugh A, assistant professor of surgery from the department of surgery and medicine,University of Colorado Medical Center in Denver Colorado as first author (101).The patients were admitted to the ICU because of multiple trauma with or without lung contusion (4 cases),abdominal shotgun wound (1) or of the chest(1),one patient had acute pancreatitis,

Although 4 patients were suspected (according to the question mark used in the table1 of the article) to have viral pneumonia, a history of viral infection was not reported.

Two patients had ingested drugs and possibly aspiration tooke place (101). Patients suffered of acute tachypnoea,hypoxaemia and did not respond to the conventional methods of respiratory therapy.

Distress developed as early as 1-96 hours after the triggering episode.

Acidosis was observed in four patients before respiratory distress developed.

Central venous pressure values were measured but results were not reported in the artcle.

The clinical and pathological features were supposed to closely resemble those observed in children with acute respiratory distress and those with „congestive atelectasis“ and postperfusion lung.

Authors found that positive end-expiratory pressure was very helpful against atelectasis ,improving hypoxemia.

Also corticosteroids seemed to be of possible help in the case of young men with polytrauma and fat embolism and possibly some cases of suspected viral pneumonia.

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A commonn finding was a diffuse alveolar „infiltration“ on chest X-ray.

None of the patients had previous serious lung disease.

Hypotension of various degree was observed in 5 patients and indicated as suffering of shock.

Four of those patients were estimated to have fluid overload from three to 7.5 liters.

Fluid overload was diagnosed in three more patients.

Chest-X-ray showed from patchy infiltrate to confluent patchy infiltrate to consolidation areas as an ominous sign predicting death.

No clinical chemistry data were given and no routine drug administration was reported.In fact, most of the attention was centered on pulmonary pathophysiology and no other data during the follow-up were given.

The possible role of the induction of positive end-expiratory pressure to avoid the hypothisized collapse of the alveoli (as cause of atelectasis) was discussed in 5 cases.

In fact, three of the five cases treated with positive end-expiratory pressure survived, compared to two of seven without this treatment.

The authors concluded that the procedure may serve to gain time for treatment of the underling process which will contribute to reverse the bad prognosis.

At autopsy (seven cases) the average weight of the lung was 1150 grams for the right and 960 for the left lung. The colour of the organ was reddish-purple as sign of blood accumulation.At that time the explanation for the massive reduction of functioning alveoli was atelectasis, in spite of the blood engorgement (sequestration) responsible for the massive increase of the organ weight.

In one case,the histological study showed engorgement of the alveolar capillaries and in case 1 intralveolar haemorrhage and edema was observable (101).

Many macrophages and **hyaline membranes** were found within the alveolar area in all but one patient.

In 1994 the first American-European consensus conference defined 4 key components to allow the definition of ARDS:

a) acutness of the respiratory distress defined as b) hypoxemia measuring the arterial partial pressure of oxygen devided by the fraction of inspired oxygen (PaO2/Fi02 less than 200 mmHg),c)bilateral infiltrates must be present on a X-ray of the chest,d) the findings should not be due to cardiac insufficiency as measured by using the pulmonary artery catheterisation (pulmonary capillary wedge pressure> 18 mmHg) .

As this procedure is not easy to perform in a surgical ICU, it fell from favor (102).

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In fact, it was removed when the „Berlin Criteria“ were decided by the Europen Society of Intensive Care Medicine (103).At that conference data set of 4.188 patients with ARDS from 4 multicenter clinical data sets in addition to data sets of 269 patients from 3 single centrs from Europe,USA and Australia with presumed ARDS, of pulmonary and non pulmonary etiology, were used.

The definition of „acute“ given to respiratory distress beginning one week or less after the triggering episode (inciting insult) was choosen to replace „acute lung injury“.It was however defined as a type of acute diffuse ,inflammatory lung injury,leading to increased pulmonary vascular permeability,increased lung weight and loss of areated lung tissue (103).

A further diagnostic criterium was bilateral chest X-ray infiltrates (as assessed by CT-scan of the chest) not fully explained by fluid overload or heart failure (103).The list also contained the respiratory criteria for definition of severity grade of the hypoxia under mechanical ventilation.

Although fluid overload (and total lung weight) is of utmost diagnostic importance and can be measured by CT-scan, there was no consensus for mentioning it in the list of the criteria for illness severity (103).

Instead, it was decided that patients may qualify as having ARDS as long as they have respiratory failure not fully explained by cardiac failure or fluid overload as judged by the treating physician using all available data (e.g. use of the ecocardiography).

In that conference it was reported that among the 4.188 patients those treated in academic centers had the worst prognosis and the lowest number of trauma patients (103).

The three categories of ARDS severity,mild,moderate and severe, could be used for therapeutic trials and eventually for resource allocation and also to decide wether to pursue ICU-treatment or not.

It was certified that according to the Berlin definition of ARDS, 29% of the patients with mild ARDS at baseline progressed to moderate ARDS and 4% further progressed to the severe form of the disease.In addition, 7% of moderate ARDS progressed to the severe form(103). It was stated that using the Berlin definition the different clinical stages (mild,moderate,severe) had significantly different increased lung weight 1371 g,1556 g and 1828 g for both lungs respectively.This parameter was not added to the definition characteristics most probably due to difficult practicability.

No special mention of a possible explanation for non-cardial fluid (blood) accumulation in the lungs can be found in the Berlin Definition of ARDS and

pathophysiology of lung weight increase continues to be disregarded at the clinical and at the autoptic level.

In fact,most of the discussions concentrated on the microscopic finding of diffuse alveolar damage (DAD) with the presence of hyaline membranes within the alveoli which has been considered to be the hallmark of ARDS and of the previous Respiratory Distress Syndrome in children and adults (*23*,104-110) independently of the etiology of ARDS.

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In a retrospective (1991-2010) pathology study applying the Berlin definition of ARDS with the addition of refractory shock (45% of the patients) and of refractory hypoxemia (11%) during the last 6 hours before death(111), hyaline membrane and the other DAD-characteristics were found in overall 45% of the cases independently of the origin (pulmonary,non-pulmonary) of ARDS .All the patients, including those with cardiogenic pulmonary edema (non-ARDS) had increased lung weight ,1693 g and 1313 g for ARDS and non-ARDS respectively.In that study an additional explanation for radiologic opacities, which could be due to atelectasis as higher inflation pressure for injecting the fixative formalin into the lung after removal ,was needed.

It remained to be explained the cause of increased lung weight (111**)** in absense of DAD .

In a further analysis of the 159 patients with the diagnosis of ARDS it was found that the exudative changes decreased with time of ventilation.In fact, 74 (90%)of 82 patients who died within one week of ARDS diagnosis had exudative changes while only 40 of 54 patients who died between one and 3 weeks after diagnosis and only four of 23 who died after more than 3 weeks after ARDS-diagnosis still had signs of exudation (112).Patients who had steroids had longer duration of mechanical ventilation and ARDS and fibrosis was more frequently of pulmonay origin. More patients were ventilated for more than 3 weeks in the scond decade (2000-2010).Thirty-eight patients(24%) had thrombossis of the small pulmonary vessels.

Unfortunately only the lungs of the 356 out of the 712 patients with approved autopsy were analysed (111,112).

Although the study gave the oppurtunity to underline the importance of using low tidal volume to prevent occurrence of ARDS in ventilated patients as suggested by the Berlin definition in the ICU it represented an additional opportunity to realize that there was more research work to be done to improve the Berlin definition of ARDS.

The cause of DAD together with the exudative changes,and microthrombosis , the great „sequestration“ of the blood in the lung vessels were not discussed (112).

In the last decades, attempts to better differentiate the etiology and clinical pictures of ARDS with the hope to individualize therapeutic efforts have been undertaken (99,113-127).

Many efforts have been invested by searching for characteristics of severely ill patients which may predict developement of ARDS recently defined as Lung Injury Prediction Score (mostly for ventilated patients) (128-138) and help to prevent it.

Among others,shock,aspiration,different traumas but also alcohol use,chemotherapy but also hypoalbuminemia and use of different drugs including furosemide,the strongest diuretic drug available, were found to be of prognostic value for development of ARDS (99).

At the same time a new approach was developed by the group of Calfee C et al.(139) by retrospectively analysing the data of 1022 patients from two previous trials the ARMA(120-122 recruting period 1996-1999)- and the ALVEOLI(123-127 recruting period 1999-2002)-study.

Authors identified two subphenotypes of patients according to their inflammatory markers.Subphenotype 2 the hyperinflammatory phenotype showed higher plasma concentration of inflammatory biomarkers,higher prevalence of vasopressor use,lower serum bicarbonate level and higher prevalence of sepsis than phenotype 1.Phenotype 2 mortality was higher than phenotype 1.The serum level of albumin was dramatically low (2.2/2.1 gr/dl) in both groups of patients .In both cohorts more than 35% of the patients had pneumonia-induced ARDS with no clear indication for the cause (bacterial or viral) of the disease.A list of the administered drugs (sedatives,vasopressors and steroids) was not given.

The article was followed by the report of a study on the treatment of patients with ARDS and sepsis with rosuvastatin as potential inhibitor of inflammation (140) and controls.The study was stopped after two-third of patients were recruted because there was no diffence in 28 days mortality between the two groups.Seventy% of the enrolled patients had pneumonia and 339 of 745 of the patients had shock at baseline.Albumin serum level and sedation drugs and the other administered drugs were not given.

In a further study (HARP-2-study),259 patients with ARDS (62% pneumonia and 41 % sepsis) were treated with 80 mg/day simvastatin and 280 were studied as control group.No difference was found in 28 mortality rate and no difference in the mean number of ventilator-free days between the two groups (141).

Only CRP-serum level was monitored as inflammation marker (141).

Bos LD and coworkers (142) studied the blood samples collected from patients with ARDS from 2011 to 2013 around day of ARDS-diagnosis.The ventilation conditions were:a) low tidal volume,higher positive end-expiratory pressure levels and prone position(severe ARDS),restrictive fluid protocol and analgo-sedation with preference for bolus versus continous administration.

Plasma from the blood samples of 700 patients was frozen at -80C and then analysed for 20 biomarkers of inflammation,coagulation and endothelial activation (142).Sixty-eight per cent of the patients had a medical indication for ICU treatment,60 % had pneumonia- and 64 % had sepsis-ARDS.Patiens had mild(37%),moderate (47%) and severe (15%) ARDS and mortality was 26%.

Based on 4 markers, Il-6,IFN-gamma,angiopoietin 1,2 (ANG1,2) two groups of patients were identified.Cluster 1 was defined as „uninflamed“ and cluster two as „reactive“.The reactive phenotype of the validation cohort had a significantly higher ICU- mortality (36.4%) compared to the uninflamed (15.6%).Interestingly, the mortality was independent of severity of ARDS based on the Berlin definition.Although the D-dimer plasma level was higher in the reactive group of patients, the difference was not higher enough to be of significant importance.Albumin-plasma mentioned among the routinely available variables was shown to have the same accuracy of the four biomarkes (shown in figure 3 of the article) as it was the case for bicarbonate plasma level.The patients of the reactive group had a lower albumin- and a lower bicarbonate-plasma-level compared to the uninflamed validation group.The albumin plasma level of the training group was even lower than that of the validation group.Authors mention that the biomarkers could be helpful to test therapeutic interventions such as

corticosteroids or macrolides.A possible correlation between the low albumin plasma levels and the increased plasma level of IL-6,PAI, ANG1,2 positive acute phase proteins could provide the explanation for the question of the cause of the increased plasma level of the 4 biomarkers.

Measurement of concentration of inflammatory mediators in the bronchoalveolar lavage from subphenotypes of ARDS (26 patients) did not show quantitative differences between „uninflammed“ and „reactive“ cohort, suggesting a difference between the systemic- and pulmonary-compartements (142)

The HARP-2-study was reanalysed as non-US study population, and furthermore on the basis of the inflammatory status (143).

For this reason additional inflammatory (IL-6- and soluble TNF-receptor-serum level) data were extracted from the patients data set.Sixty-one % of the patients in the hypoinflammatory group (353 patients) had pneumonia, compared to 43% in the hyperinflammatory (186) group.Eighty-one per cent of the hyperingflammatory group needed vasopressors and creatinine serum-levels in the patients of this group were significantly higher (1,7 mg/dl) than that in the hyppoinflammatory group (0.85 mg/dl).The authors found that simvastatin improved 28 and 90 day survival in the hyperinflammatory group.

Lawler PR and Fan E (144) suggested to consider the results of the restrospective non randomized study as exploratory and hypothesis-generating for prospective studies as it was also suggested by Heijnen et al (145).

A critical review of the latest literature about the actual value of subphenotyping of the various ARDS-patient cohorts concluded that prospective consistent identification of ARDS-subphenotypes are needed to better understand the underlying biology before one can decide to test which therapy may be more appropriate for the different cohorts (146,147).

A research workshop,by underlying the necessity to better identify subphenotypes of ARDS-patients concluded that the search for new effective treatments has been unsuccessful because of the heterogeneity of the causes and the biology of syndrome. It seems however appropriate to add measurement of blood volume and normalisation of albumin serum level to the list of the additional treatable traits (148).

Based on these experiences a study was performed on SARS-CoV-2 positive patients treated in the ICU with 20 mg atorvastatin/day.Twohundred-neinty patients were assigned to the atorvastatin, 297 to the placebo group,then followed for 30 days.The median lenght of ICU-stay was 5 days.Ninety-two percent of the patients recieved corticosteroids.Mortality was 31% and 35% in the atorvastatin and in the placebo group, respectively.The median duration of ventilator-free days was similar.Venous thrombosis frequence was similar as it was frequence of renal replacement therapy (149).

The question still remains what causes lung „edema“ as supposed by imaging and by histopathology, especially when pneumonia is the primary diagnosis ,which is accompanied by sepsis and by the need for vasopressors.

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A further prognostic marker introduced by recent publication could be the following: Not all patients with ARDS present the classic histologic changes, namely diffuse alveolar damage (DAD) and presence of hyaline membranes (111,128) in the alveolar wall in close contact with the capillaries.Those patients without DAD may have a better prognosis . A suggestion to modify the definition of ARDS has been published recently to differentiate respiratory distress due to diffuse alveolar damage (DAD) and that without DAD.

An attempt has been also made to further characterize the causes of the clinical situations leading to the developemnt of hypoxia accompained by the typical radiologic findings of ARDS resulting in the histologic picture of DAD.

While „conservative“ fluid administration is suggested in critically ill children(150) and in adults with ARDS because of the fear of more fluid entering into the alveoli (151) ,the dilemma of this decision is influencing indication for mechanical ventilation and survival of many hospitalized patients, as the need for it is also influenced by blood pressure (152).

Although many autopsy-studies of patients who died with ARDS report a two- to 5-fold increase of the weight of both lungs, they mainly concentrate on histology findings especially on DAD,presence of hyaline membranes and microthrombi in the capillary, the question of the sequence of events however is seldom addressed (153-159).

In spite of the fact that DAD,hyaline membranes and microthrombi are not specific for COVID-19 victims (160) and that viral particles are not diffusely found in the lung at the microscopic level,the possible link between the need for vasopressor administration due to hypotension, the endothelial and hypoxic tissue damage (161) and and the early apparence of DAD has not been discussed so far.

Hypotension due to hypovolemia also explains the quite frequent presence of tubulus necrosis in the kidney, also frequently found in SARS-CoV-1 (162), MERS-(163)and COVID-2 victims (164).

Hypotension due to hypovolemia may also be the explanation for

the disturbed sublingual microcirculation observed in severely ill ICU-patients (165-167) as it has been observed in severely il patients infected with the influenza A (H1N1) -virus (118).In a further attempt to reclassifying ARDS these findings were not considered, but lung functional and radiologic parameters were the basis for the new definition (168).

This continues to be the case in a recently published effort to reconsider the pulmonary changes occurring during development of COVID-19- pneumonia (169); a new definition of the COVID-19-ARDS has been suggested namely CARDS.Patients were devided in 5 groups according to the revised Berlin definition of ARDS:a)non-CARDS,b) mild CARDS,d) mild-moderate CARDS,e) moderate-severe CARDS and severe CARDS according to their PaO2/FiO2 value (from >300 mmHg to<100 mm Hg).The study concentrated on lung parameters including lung weight.

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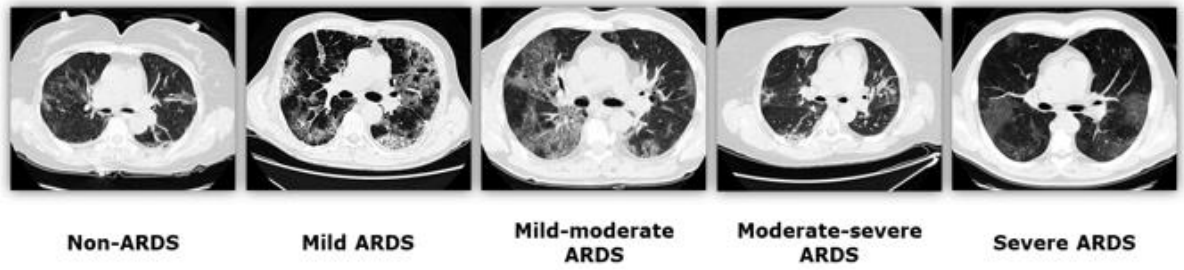

Figure S3: lung Computed Tomography (2-3 cm below the carina) extracted randomly from the non-ARDS, mild ARDS, mild-moderate ARDS, moderate-severe ARDS and severe ARDS. As shown, it is hard to find a relationship between the severity of the imaging and the severity of the hypoxemia.

Fig.*4 CT-scan oft he CEST of patients with ARDS of different severity(from 169 with permission).Only at a closer look opacities („shadows“) can be detected in the CT-scan of the patient with severe ARDS.*

Surprisingly the D-dimer plasma levels reported were measured at baseline (overall range of 140 patients=215-411 ng/dl).The plasma levels of D-Dimer did not vary significantly within the different severity groups(169).The changes of D-Dimer plasma levels in the 14 patients (of 140) who died were not reported but there was no significant correlation with outcome .In this study the CT-scan result did not correlate with hypoxemia (Fig.4).Contrary to the literature data concerning the increase of lung weight proportionally to the severity of ARDS,

in CARDS-patients the lung weight in moderate-severe and severe cases was 50% of the weight reported for non COVID-ARDS.This findings are surprising as the CARDS fulfill the Berlin definition criteria on the one hand and,on the other, the autoptic data also do not allow a differentiation between „pneumonias“ of different origin.

Only the total lung stress and not the lung weight is correlated with negative outcome.

Death occurred only in mechanically ventilated patients.

The conclusions of the article were critically commented by Neetz B et al. (170)

Furthermore,in most of the autoptic studies published so far, a multy-fold increase of lung weight has been regularly found in hospitalized COVID-19-patients who died of the disease. It remains therefore difficult to allocate the groups of patients with different oxygenations (PaO2/FiO2 from226 to 83 in moderate-severe to 65 in severe ARDS) and significantly different FiO2 but no different pH. It remains also difficult to understand the indication for mechanical ventilation, 4 to 9 days after admission, which was justified by the progression of respiratory distress.

The list of 34 different reasons reported by the attending physician who decided mechanical ventilation in 34 patients does not contribute to better understanding.Respiratory rate was 30 and above in 9 of the 10 patients where this information was reported but these numbers can not be found elsewhere in the tables of the article.

Quite often „agitation,intolerance“ to CPAP or NIV or delirium were reported as an additional reason for the intensivologist to indicate mechanical ventilation at different times

after hospitalisation.Information about drugs administered before patients were admitted to the high-dependency unit (e.g.high dose corticosteroids and /or diuretics) and other supporting measures could help to better understand the peculiarities of this „subgroup“ of COVID-19 patients (age range 51-69 year) especially the changes of the esophageal pressure swing.Also administration of 6 mg dexametasone may be responsible for uncontrolled behaviour of the patients under certain conditions.

Dehydration could also have contributed to the „changes“ of mood and cognition (171)

Only septic shock was one of the reasons, which allows to think of a primary cause outside of the lung.In a second patient acute kidney injury was mentioned together with agitation during CPAP, PaO2/FiO2 77 mmHg.

It remains difficult to think about the cause of death in the 14 patients, who died in the San Paolo Hospital in Milan without an anatomical correlate, if one does not have additional clinical data including blood volume together with serum albumin concentration and the list of the drugs administered before and during mechanical ventilation. Autoptic findings would also be of great help.

By measuring sublingual microcirculation for vessels smaller than 20 micrometers in 12 severely ill COVID-19-patients Damiani et al. (167) found an inverse correlation between the plasma level of D-Dimer, the perfused vessel density and the PaO2/FiO2-value (205). In that study D-Dimer plasma level ranged between 717 and 5536 ng/ml.

An inverse correlation between plasma level of D-Dimer and microcirculatory changes was found also in the retinal vessels of severe COVID-19 infected patients(172,173).This indicates once more that the changes observed in the alveolar capillaries and those observed in other territories (174) may be attributale to the same noxae, namely dehydration conditioning tissue oxygenation followed by the emergency induction of the perivasal clotting process.

The „conservative“ fluid administration also influences the nutritional part of the care of the hospitalized and especially of ventilated COVID-19-patient.In fact,in the latter case ,intensivists are reluctant to administer calories and aminoacid solutions intravenously because of the possible volume overload.

On the other hand ,vasopressors to sustain systemic circulation and diuresis are administered regularly in severely ill patients (139).

In addition,these vital questions are discussed without considering that normal albumin serum level (3,5-5 gr/dL) is essential for assuring normal blood volume,to guarantee normal erythrocyte circulation, especially in the alveolar capillaries. This aspect of the clinical picture in severly ill patients at the ICU has not been considered when therapeutic options were discussed in ARDS-subphenotypes (139,143).In the hyperinflammatory group, in fact, systolic pressure was under 90 mm Hg and the use of vasopressors was frequent.The discussion about liberal or conservative fluid support can not be productive if albumin serum level is not considered (149,150,166).

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Conservative fluid administration is routinely associated with furosemide administration ,to assure sufficient urine production.This strategy however leads to hypovolemia nad hypotension wich brings into play vasopressors in the attempt to reduce hypotension.Vasopressors however can even worsen the ischemic consequenses in the tissues be responsible for the local release of acute-phase cytokines (e.g. IL-6) by the recruited macrophages and for the local and hepatic production of chemokines (e.g. IL-8) (58) and other acute-phase proteins.

Under normal conditions, a person of 70 kg body weight needs about 2.900 milliliter of water every day.About 700 ml of this water is necessary to preserve the Alveolar Lining Fluid (Table) and to avoid dryness of the mucosa of the upper respiratory tract. Every day that amount of fluid is expired with the air.

TABLE:Physiological functions of Alveolar Lining Fluid and consequences of its reduction

ALVEOLAR LINING FLUID (ALF):A PROTECTION LAYER

-liquid sheet of 0.2 um height

-total amount in the lung alveoli = 36 ml

-it covers the surface of the alveolar epithelial cells (AEC I,II,0,1-

02um)

-it contains surfacant protein B (SP-B)* produced by AEC II.SP-B

avoids the collaps of the alveolus by reducing the surface tension

at the air-liquid interface.

-it avoids desiccation of AECs due to direct exposure oft he cells to air

-it is continously maintained by water supply from the capillaries

and/or interstitium or

-by fluid reabsorption from the apical side oft he alveolar epithelium

to the interstitium and drained by the lymphatic system.

-it protects from particles,irritants and pathogens

-it constitutes the enviroment oft he alveolar macrophages

-it contains defensines

_____

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ALVEOLAR LINING FLUID (ALF): A PROTECTION LAYER (continuation)

-modification of the hight and composition of the ALF

will impact the rate of O2/CO2-diffusion

-reduction of O2-diffusion first induces an „ischemic“

Reaction and recruitment of inflammatory cells which will then worsen hypoxia

-reduction of SP-B* induces an increase of pressure on the capillary and can induce a collaps of it

- riduction of the mount of fluid leads

to a damage of the AECs on the alveolar side and to

a shrinkage of the endothelial cells on the capillary

luminal side.

-The latter allows plasma to invade the

alveolar lumen and triggers the „provisional clot“-

formation (hyaline membrane) as a protective measure to avoid further extravasation of plasma into the alveolus

-inflammatory reaction futher reduces gas-exchange

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*SP-B=surfactant protein B

This water originates from the alveolar capillary fluid which is kept constant, indicating that the fluid volume of the alveoli is replaced about 20 times/day (175).

To understand the possible cause of the changes occuring in the alveoli before DAD develops , it is important to recall the anatomical and functional peculiarities of the lung.

As the kidney needs water to exert its function as well as the brain needs sugar,the lung needs water and oxygen (176) to fulfill ist function.Oxygen from the inhaled air is transferred to the erythrocytes through the wall of the alveolar capillaries replacing C02 which will be expired.To this aim alveolar tension has to be reduced to 0.

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Similarities between the hepatic and the pulmonary circulation

The circulation of the lung resembles that of the liver.While the venous (portal circulation) blood reaching the liver transports nutrients through the venous blood from the intestine and hormons from the pancreas to the liver reprsenting about 70% of the blood ,the remaining 30% of blood flows into the liver from the hepatic artery.The wall of the liver sinusoids is anatomically predisposed to allow quick passage of nutrients from the intestine through the fenestrated endothelial cells (sieve plates) and to be quickly taken up by the hepatocytes.The wall of the sinusoid is made of the prolongations of specialized mesenchymal cells (stellate cells) underneed the endothelial cells which however do not obstaculate the close contact between the fluid content of the sinusoids and the hepatocytes.

The tissue macrophages however are located inside the sinusoidal lumen while the alveolar macrophages are outside the vessel but inside of the alveolar lumen to eliminate foreign bodies which eventually surpass the bronchial defense apparatus.

When continous liver damage occurs, liver cirrhosis may develop and portal blood flow through the liver is strongly reduced. Portal hypertension is the consequence.

In this case, different amounts of venous blood are sequestered in the venous portal system as only a part of the portal blood can reach the cava trough venous collaterals (e.g.esophageal varices).Reduced liver function and reduced albumin serum level,reduced systemic blood volume and ischemic damage in the intestinal tissues are the consequence.

Administration of diuretics may reduce blood volume and blood pressure causing prerenal kidney insufficiency leading to the so-called hepatorenal syndrome.

In the lung venous blood from the right heart is transported by the pulmonary circulation,the pulmonary veins, to the pulmonary capillaries which bild the borders of each alveolus.The second circulation is represented by the bronchial circulation originating from the aorta and from the intercostal arteries.

The pulmonary capillary vessels are much thinner than conventional arteries and their wall is so thin that fluid (see above) and gas can move across.

The capillary network of the alveoli builds a „sheet“ which allows to reduce flow resistance and favours gas exchange.The availability of the capillary segments may vary acccording to the transmural pressure difference between inside and outside of the vessels.

When this is slow many capillaries are closed.They may however be rapidly recruited to satisfy increased bloood flow (low pressure high flow system).This flexibility is necessary as the lung can not control the cardiac output.When the capacity of the heart to pump venous blood into the lung is reduced, splancnic stagnation of venous blood occurs.

When pulmonary congestion develops a reduction of blood volume takes place and the heart (right and left) has less blood volume to pump and change of the heart size may not be apparent at X-ray-study.

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In the liver, when metabolic needs of the body increase, as it is the case when the need of acute phase proteins becomes an emergency, more „inactive“ hepatocytes are „recruited“ and the production of proteins in the hepatocytes within the liver lobule dramatically increases.To this aim an increase of aminoacid delivery trough the portal blood into the liver sinusoids takes place.

ARDS-experimental models (pulmonary hyaline membrane disease)

Many publications dealing with experimental acute lung injury (ALI),a definition used as a synonym of ARDS and having the DAD (inflammatory infiltrates,thickened alveolar septae and deposition of hyaline membranes) as histological basis, have been pulished in the last decades (177-181).

However no animal model was considered to reproduce all the pathologic features of human DAD which is characterized by: a)an early exdutative phase accompanied by accumulation of neutrophils in the vascular,interstitial and alveolar spaces („neutrophilic alveolitis“),b) deposition of the so-called hyaline membranes due to the deposition of fibrin and other proteinaceous debris at the alveolar site, as a sign of the disruption of the alveolocapillary membrane,c) interstitial thickening and d)formation of microthrombi interpreted as evidence of endothelial injury and activation of the coagulation cascade.

The lack of reproducibility of the human ALI has been justified by the fact that the human pathology may be caused by a combination of factors which can not be reproduced in animal models (177-181**)**.

Hyaline membrane formation (Figures 5, 6) was not in focus any more (97,101).

All the experimental models however concentrate on the functional lung damage and disregard the systemic consequences of the endotoxin administration caused by reduction of fluid and food intake resulting in blood distribution abnormalities and decreased glomerular filtration (179-181) or by the changes of gene expression not only in the lung but also in the organs including the liver (182).In those experiments expression of smooth muscle alpha-actin was reduced not only in the heart but also in the lung 24 hours after endotoxin administration in a dose-dependent manner .This has to be taken into consideration whenever lung myofibroblasts are studied using alpha-SMA as marker antigen(183) in damaged lungs of patients who died at different times after SARS-CoV-2-infection.

Also the decrease of CD31 (PECAM-1) gene-expression in the inflammed areas is most probably due to the local production of TNF-alpha,which downregulates PECAM-1-gene-expression in endothelial cells(184).

The apparence of fluid in the alveolar space due to changes of the capillary permeability is called „pulmonary edema“definition which however generates a clinical prejudice as if it were due to simple diffusion classically happening in the case of cardiac overload when, contrary, to the ARDS situation, reduction of fluid administration and administration of diuretics are responsible for reduction of blood volume through the capillaries. Administration of fluid and albumin is essential to reestablish normal blood volume and normal oxygenation.

1941 a pivotal report was published by Harry A Davis (185) from the department of Surgery of School of Medicine of Luisiana State University and Charity Hospital of Luisiana at New Orleans in Archives of Surgery.He reported about the clinical and autoptic findings of a 42 year-old male patient who was admitted to the hospital because of intestinal obstruction and massive dehydration due to severe and frequent vomiting.The patient was clearly in a state of circulatory failure with a systolic pressure of 75 mmHg and a pulse rate of 120 per minute.The patient died four hours after admission.Besides a 28 cm lenghth of dark red strangulated jejunum with signs of gangrene all the other viscera except the lung were reduced in weight.The lung was heavy and moist and numerous petechial hemorrhages were present in the visceral pleura.Microscopically the alveolar capillary were markedly distended and filled with closely packed red blood cells.

Furthermore the alveoli were filled with fluid and extravasions of blood were present in many areas(185).

This experience stimulated the author to perform a study on seventeen dogs (6 dogs were studied as control group) with a body weight between 5 and 10 Kg.Twenty-five cc of sodium chloride/Kg body weight (25%) were injected subcutaneously into a hindleg.

Two or three hours after injection the blood pressure began to progressively decrease accompained by a progressive increase of marked edema at the site of injection until death which occured between 5 and 10 hours after injection.

Outside of the edematous area of injeced muscle on the subcutaneous tissues elsewhere were dry and sticky.

While the left ventricle was empty, the right ventricle was filled with thick partially coagulated blood.

While the pleural cavities were dry the lungs were brick- and purple -red showing numerous petechial hemorrhages in the visceral pleurae.The surface of the lungs exudated dark venous blood after cutting.The alveolar capillary were dilated with closely packed erythrocytes many of which were found in the capillary spaces as a result of the rupture of the capillaries.

Aveoli were filled with clear acellular fluid.Te extent of the alveolar damage was correlated to the length of the shock situation.

The animals which died quickly had less alveolar fluid(edema) than those which survived longer to the hypotension.

In a few animals some macrophages were detectable in the alveoli.

Davis concluded that dehydration is sufficient to explain the changes observed,most strikingly in the skin,the lungs and the weight reduction of the other organs and that there was no need to suppose a role for „toxic“ substances.

He supposed instead, that hemoconcentration is accompained by a great reduction of oxygen content in the blood.

First viral acute Respiratory Distress Syndrome as post-influenza „pneumonia“

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There are similarities of the macroscopic and clinical changes described by Davis as a consequence of dehydration with those observed by Petersdorfer et al.(23)1959 in the lungs of patients who died of „post-influenza pneumonia".

While the number of emergency clinic visits for non-respiratory illness remained constant,the number of respiratory illness increased sharply with a peak during the last two weeks in October when 170 and 157 were seen (23).It was the year 1957 when the asian influenza epidemic arrived in New Haven in late september .Most of the infected persons had mild disease but 91 of those patients with pulmonary infiltrates, who were seen at the emergency facility of the New Haven Hospital were hospitalized during the six weeks period from October 1957.This was a six-fold increase of the hospitalisation number compared to that of the previous year.

Among the 53 male,26 were older than 50 whereas 24 of 38 female were between the ages of 16 and 40.Authors commented these numbers saying that

old men were prone to develop pneumonia following influenza while female were most vulnerable during the child-bearing period.

Forty of 91 hospitalized patients were black, while only 15% of the medical ward patients were black.Bacteriological investigation demonstrated bacterial infection in 38 patients while the other 43 patients with radiological or autoptic

signs of „pulmonary infiltrates" were diagnosed pneumonia of undetermined etiology.Ten patients were diagnosed as having an acute tracheobronchitis.

Virological diagnostics consisted in investigating blood samples for the hemoagglutination-inhibition test and throat lavage fluid for virus culture.

A fourfold increase of the hemoagglutinin-inhibition test was found in the blood

of most of the patients who were tested, independently of the supposed cause of the pneumonia suggesting that the asian-influenza virus was causal factor in most of the patients.

Symptoms preceeding the visit to the emergency room were quite similar whereby ninety per cent of the patients had fever from 38 to 39 degree celsius.

In neinteen patients the body temperature was even higher at presentation and persisted 48 to 72 hours after admission.

Patients with comorbidities such as heart (including three patients with hypertension)- or chonic lung diseases,diabetes and alcoholism were found to be more susceptible to post-influenza pneumonia as it was the case for 10 pregnant women.Only 36 of the 91 patient were free of comorbidities or pregnancy.Eleven of the 91 patients died.Four of them (68,72,76 and 77 year old) were suffering from chronic diseases, the pneumonia played an important causal role in their death.The other seven patients who died were young healthy adults who succumbed to an infection characterized by: acute onset,high fever,severe dyspnea,leukopenia , bloody sputum,anoxia and circulatory failure.Autoptic findings in a 19 year-old student who died 48 hours after admission are as actual (186) as

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pathognomonic.The macroscopy of the lungs showed edematous red organs with a weight twice the normal.Microscopy showed severe hemorragic pneumonia with necrosis and **hyaline membrane formation** (fig.5).The picture was very much similar to that

observed by Berfenstam et al.(101) in the lung of rabbits intoxicated with O2(Fig.6).

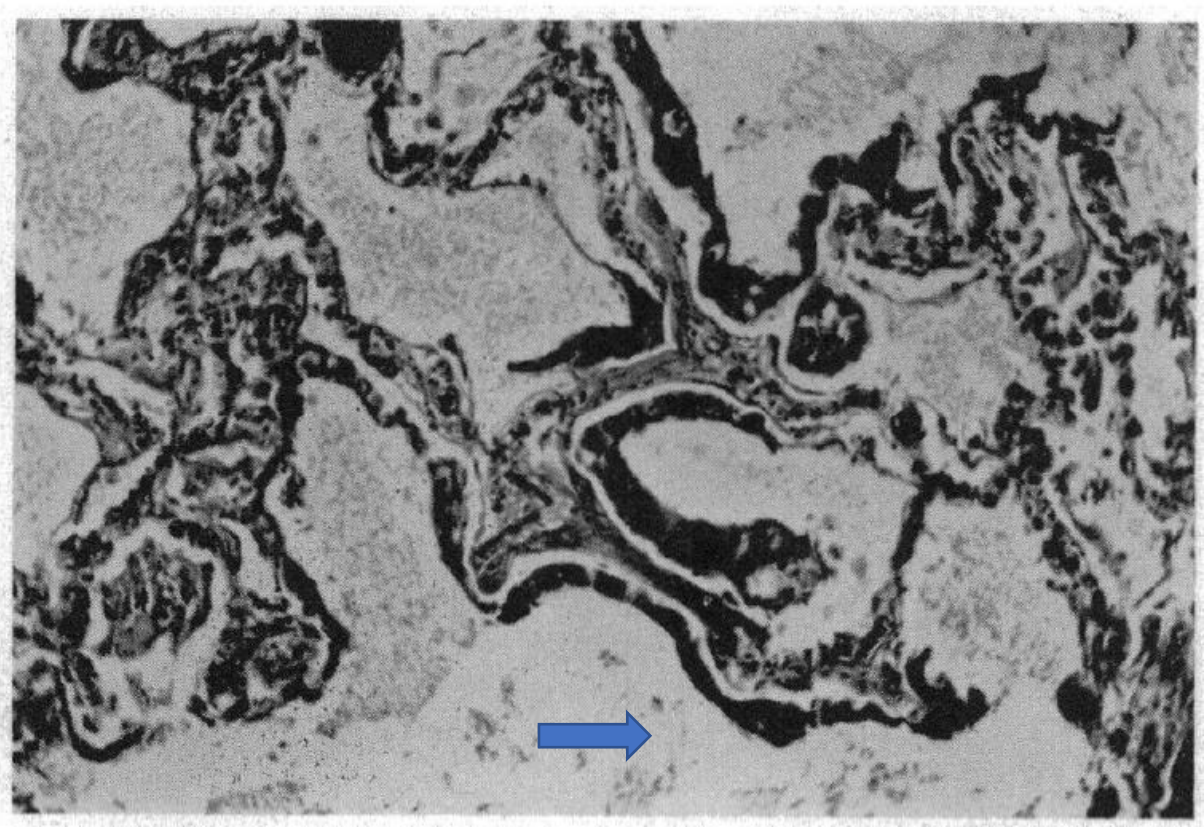

*Fig.5.Alveoli lined with **hyaline membrane(arrow)**.Modified from Petersdorfer et al.1959(**23**).With permission.*

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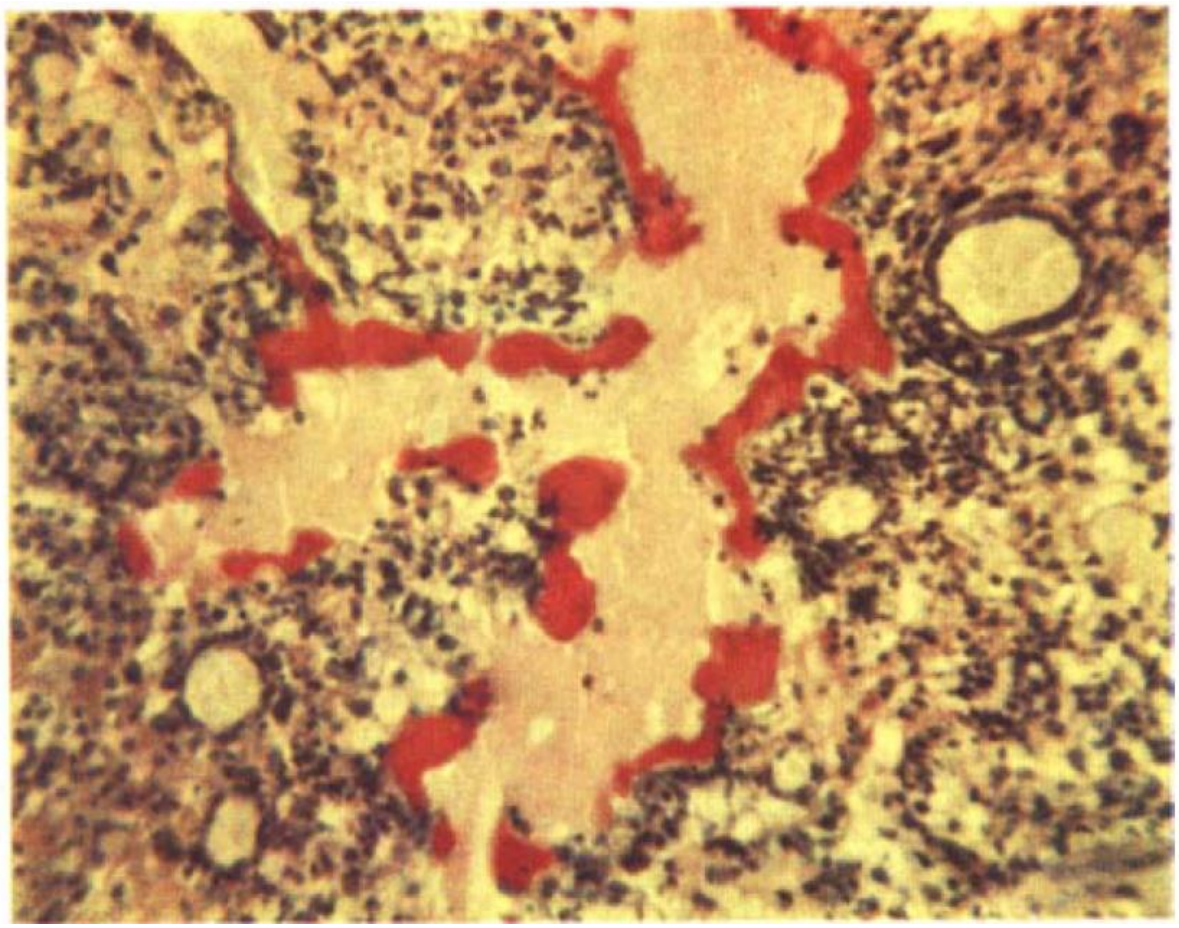

Fig.6 Hialyne membranes (periodic acid- Shiff staining) in the lung of a rabbit treated with oxigen.(from 101 with permission)

All the patients showed a similar clinical pattern with mild upper respiratory dissturbances, systemic symptoms lasting for several days followed by fever,cough, dyspnea,bloody sputum and leukopenia.Authors undescored the fact that the pathologic picture consisting in acute tracheobronchitis, diffuse involvement of the pulomonary parenchyma,necrosis of the alveoli,hyaline membrane formation with acellular hemorrhagic exudate was quite uniform not only in the New Haven patients but also in those who died of the same disease in USA and in England.

This report suggests many similarities with the actual pandemic situation and also describes similar autoptic findings with the central histologic hallmark, namely the presence of the hyaline membranes , which were known to represent fibrin deposition (97-99) and has been reported not only in premature children in the placenta ,in the lung of O2-intoxicated guinea pig and rabbits (97,101) but also in human adults (102,103).As it was described by Fujikura (97) fibrin deposition can be observed not only in the lungs of newborns but also in normal term placenta(97) perivillous fibrin deposition in the placenta of COVID-19-positive pregnant women after delivery has been recently reported together with throphoblast necrosis and histiocytic intervillositis .The triad has been called SARCOV-2 placentitis(187).

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Respiratory Distress Syndrome (pulmonary hyaline membrane disease becomes Acute Respiratory Distress Syndrome (ARDS)

Description of ARDS -cases by Ashbaugh and collegues (94) and later in several other reports of ALI/ARDS-cases (132-146) are strikingly similar.

In an article dedicated to clinical and radiologic feature of pulmonary edema Gluecker and coworkers thouroghly descibe the clinical characteristics of the „PERMEABILITY EDEMA WITH DAD and clearly descibe the corresponding radiological pattern as detrmined by conventional X-ray or CT-scan of the chest (188).

It is clear from the biginning that this part deals with the clinical picture of ARDS,term used for acute or subacute pulmonary „lesions“ accompanied with hypoxemia.

It has to be made clear that: a) the lesions observed at radiography are not caused or influenced by heart failure and

b) ARDS occurs without an increase in pulmonary capillary pressure.

Authors devide ARDS in two major groups depending on the possible different etiologic mechanisms which may lead to their development:

a)ARDS due to an underling (assumpted) pulmonary disease and b) ARDS secondary to extrapulmonary disease which manifests with an interstitial edema and alveolar collapse.

Authors justify the differentiation with the different implications for

the treatment of patients for example in the cases of sepsis,acute pancreatitis,severe trauma, or blood transfusions compared to that due to a more directly induced damage of the alveolar and vascular endothelium of the lung resulting from the exposure of the cells to chemical agents,infectious pathogens (such as bacteria or viruses),gastric fluid or toxic gas supposed to destroy or severily damage the tissue.

ARDS may undergo three stages

a) the first stage is characterized by interstitial „edema“ (exudative) with a high protein content that rapidly fills the alveoly and is associated with hemorrhage and formation of hyaline membrane.

*The words **exudative , edema or pneumonia** are not radiologic findings but correspond to a deduction which is not always possible at the time the radiologic investigation is performed.The radiologist can only speak of groung glass opacity(GGO) withuot an apparent cardiologic cause.*

Furthermore there are early cases of ARDS without DAD(characterized by an „inflammatory“ infiltrate) which should not be called pneumonia.

One important differenzial marker between GGO of cardial and non-cardial origin, is the lack of the Kerley lines characteristic of increase of interstitial fluid of cardiac origin.

b)the second stage (proliferative) is characterized by the organisation of the alveolar fluid (called exudate).

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c)the third stage is characterized by formation of fibrotic septa.

At CT-scan, ARDS has a more peripheral and cortical distribution which may change by changing the position of the patient suggesting that atelctasis plays also a role in the inhomogenous (regional) apparence , which is more common in ARDS than in classical bacterial pneumonia.

It is intersting to learn that recurrent „exudative“ episodes can occur in the proliferative and fibrotic stages of ARDS resulting in a mixed radiologic picture (Fig.7)

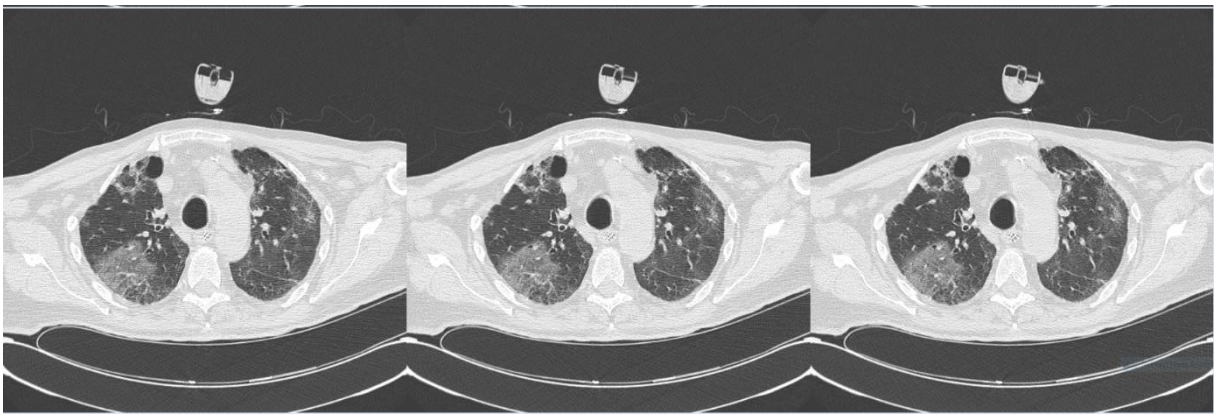

Fig.*7. CT-scan of the chest of the same patient described above.The study was performed three mounth after discharge from the intensive care unit.It shows a mixed radiologic picture as a result of ARDS and of two mounths of mechanical ventilation.Patient is now suffering of long-COVID 18 mounths after release from ICU.*

Modern development of ARDS: combination of new imaging techniques and pathology.

In the last 20 years many efforts have been invested in using CT-scan of the chest as a further prognostic characteristic.Recently a Radiographic Assesment of Lung Edema (RALE) score has been introduced (189) to assess the extent and density of the pulmonar opacities in patients with different severity grades of ARDS (190).Also by using this method there was a positive correlation with more conservative fluid therapy , lower RALE-score and mortality.

The APACHE III-score (191) has been used to differentiate two groups of critically ill hospitalized patients partecipating to the different therapeutic forms,conservative versus liberal, based on clinical characteristics.

It has to be pointed out however that the clinical value of two very important parameters namely albumin and creatinin serum level were quite underestimated as only albumin serum level of 2.4 gr/dl scored 6 points and creatinin of 2.2 mg/dl 7 points of 107 total points while an albumin serum level of 2.8 gr/dl scored 0 points and a creatinin serum level of 1.8 mg/dl scored 4 of 45 total points.On this basis is difficult to estimate the effect of different fluid administration policies in patients who were evaluated because of increased amount of fluid in the lung (called edema) without knowing the blood volume.

Recently two dimensional (2D) and three-dimensional (3D) reconstruction of pulmonary vasculature by using CT-scan of the chest has been used to study changes of the vascular morphology (192) or the loss of vessels in smokers who had undergone lung resection because of lung cancer (193).Combination of histological and imaging data allowed the 3D-riconstration of the pulmonary vasculature down up a vascular radius of 0.5 mm and to establish a correlation between changes of the vasculature as assessed by histology and as measured by CT-scan.

By means of new software, retrospective analysis of CT-scan of the chest allowed a 3D reonstruction of the pulmonary vasculature and to calculate the total volume of smaller pulmonary vessels including those with a cross-sectional area below 5 quadrat mm(BV5) in persons exposed to air pollution.It was found that higher exposure to ozone was associated with smaller vascular volumes of the small pulmonary vessels (194).

Recently a combination of hierarchical phase contrat tomography (HiP-CT) and pathology techniques has been used in 5 intact human organs and in a lung from a deceased donor infected with HuCov-2, to study regional changes in tissue architecture of the lung (195).The study of the lung of a 54 year old aptient who died of COVID-19 allowed to differentiate parenchyma deterioration in COVIDs(less deteriorated) and COVIDc(more deteriorated).

Although ARDS was supposed as cause of death in the infected patient, the available lung portion were the upper left lobe and a biopsy of the right upper lobe of the mechanically ventilated patient.

The classical histologic finding of ARDS was not described.

The same technology has been applied by Eckermann et al (196) in the analysis of 6 lung samples from patient who died of respiratory failure due to COVID-19.

A three dimensional representation of the classical findings of DAD was generated with impressive pictures of hyaline membranes adjacent to the epithelial lining,moderate lymphocytic infiltration and singular thrombi in small pulmonar veins were described.

Also samples with high amount of swollen,inflamed blood vessels and thick hyaline membranes were found.In some slices capillaries filled with erythrocytes were shown.

Although in the manuscript the macroscopy of the lungs was not mentioned all patients had havy bluisch, firm lungs with impressive congestion of the capillaries filled of packed erythrocytes (197). All patients died after different time after HuCoV-2 infection, presence/proliferation of viral particles however was not described.

It is infact important to realize that HuCoV-2-infection is the **trigger** of pathophysiologic changes,which may not be later attributed to direct influence of the virus (198).

While there was no difference in the CT-characteristics at the hospital admission between the group with moderate type and the severe or critical type HuCoV-2-infected patients (88% had GGO), a difference could be observed in the second CT-scan of the severe/critical group which showed more frequently a lower (50% vs 82%) occurrence rate of ground-glass opacitity (GGO), but a higher occurrence of crazy-paving pattern (75% vs 39%) and of consolidation (81%).The occurrence rate of pleural thickening or adhesion also picked

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(100%) at the second CT-scan(199).Similar results were reported for the groups of the moderate or severe/critical patients at repeated CT-scans (200) by Huang Y et al.

Study of changes of pulmonary vascular volume during hospitalization may serve as biomarker of abnormal gas tranfer after HuCoV-infection.

In fact, a switch from BV5 to BV5-10 or above BV10 was observable in patients with low diffusion capacity of carbon monoxide (low DLCO).

Measurement of diffusion capacity may become the most important fuctional test after HuCoV-infection resolution(201) .The report further underlines the distribution change from the smallest to the larger capillaries/vessels.ll

How SARS-CoV-2-infection of the nose leads to cardiac arrest and death

Autopsies at the initial stage of the disease including analysis of the upper respiratory tract are seldom performed (202).

The autoptic findings did not show particular damage at the level of the organs other than the lungs (203-208) while the changes of the mucosa of the upper respiratory tract were not studied.

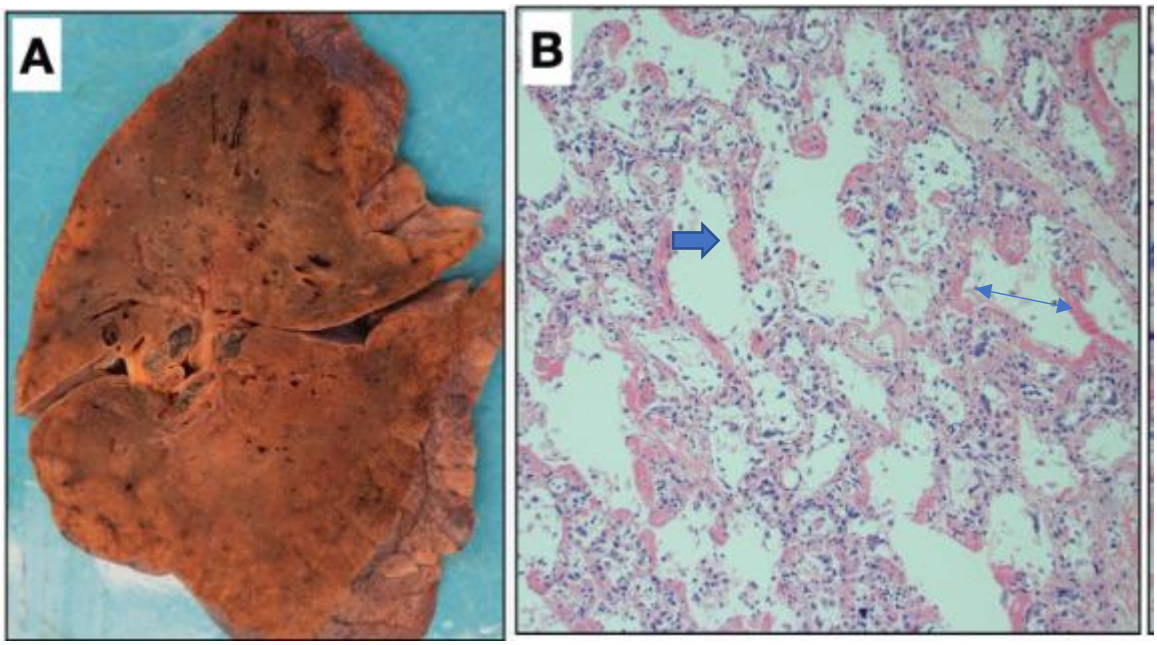

Fig.*8 Macroscopic picture of a lung taken at post-mortem autopsy of a patient who was admitted at the Mount Sinai Hospital and died because of HuCoV-2-infection.The gross appearance resembles that of a solid organ with „patchy" areas of consolidation(A).The microscopic picture (B) shows the „congestion" of the alveolar capillaries,which may represent the initial episode of the lung changes accompained by deposition of hyaline membranes (blue arrows) and liquid outflow from the capillaries into the alveoli most probably induced by dehydration.The pictures were taken from figure 1 of the article published by Bryce C et al. in Modern Pathology 2021;34:1456-1467(202).*

The gross morphology as shown in figure 8 (panel A) and histology (panel B) of the lung regularly found in hospitalized patients who died after SARS-CoV infection, was very much

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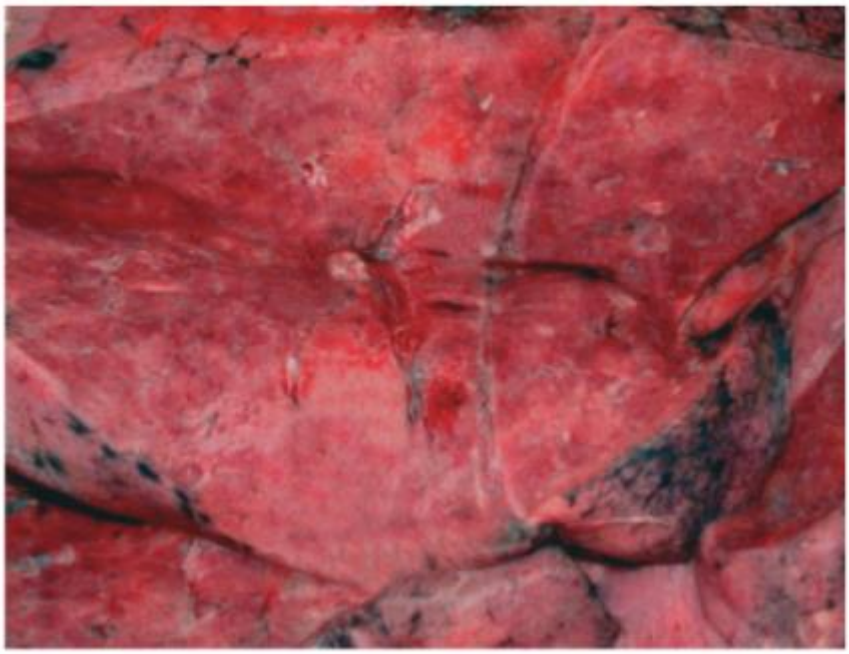
similar to that observed in the victims of the SARS-CoV-1 (158) as shown in figure 9.The macroscopic picture oft he congested lung and microscopic picture of DAD with the classical hallmark of the presence of hyaline membranes was also very similar.



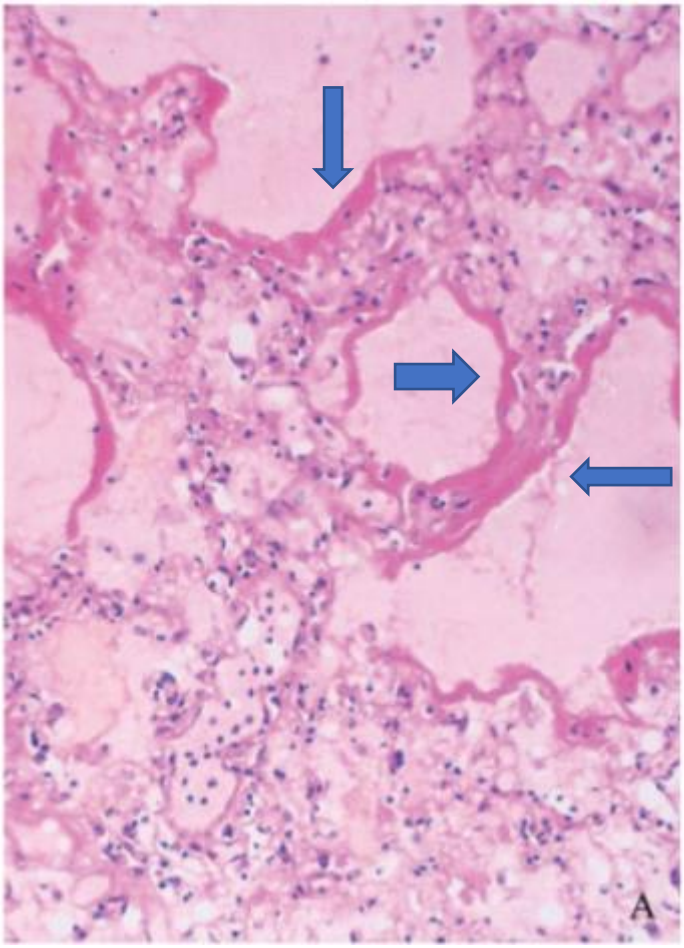


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Fig.9.*Gross morphology (upper panel) from the lung of a patient who died after a SARS-CoV-1-infection shows a congested lung while the histology picture (lower panel) shows the hyaline membranes (blue arrows).Modified from Ding et al.(158)*

As acute kidney injury is a clinically ominous prognostic sign (50). It is of relevance to note that tubular necrosis as „marker“ of hypovolemic shock has been often found (205-207) in absence of viral replication.

In fact, viral infection has not been found in the kidneys although positive PCR-findings were described and viral particles were suggested(205) when electron microscopy technique was used, which however needs a long experience to check plausibility(207,208).

Ischemic signs were not only found in the kidney but also in the heart and in the brain (209).

Multifocal vascular injury but no viral particles was described in the brain of 13 patients who died (11 of them died suddently and unespectedly (209) Microinflammatory changes have been also described in the heart of 6 patients who died of COVID-19 (210) and also in the retina (211).

In situ-hybridisation using SARS-CoV-2-specific-RNA gave negative results.

In one other study,although in situ hybridisation and immunohistochemistry studies gave positive results for the presense of viral „material“in cells of alveolar capillaries,viral particles were hardly detectable in those structures (212)

Multifocal areas of retinal microvascular ischemia associated with multiple bilateral cotton-wool spots have been described in the left eye (OCT-angiography) of a patient who developed pneumonia and was tested positive for COVID-19 (day 17 of illness) and the authors concluded that retinal microangiopathy can develop in the late-phase of SARS-CoV-2-infection and that similar changes „can be happening in other less-accessible organs“(211) Gelsomina Mansueto stated in a short communication (213) „ The lack of autopsy findings did not allow us to evaluate with greater serenity what was happening and to understand that the specific pulmonary histological pictures and the secondary multiorgan damage in the patients with comorbidities were very similar to those already observed for other viral infections“.She also underlines in her conclusion the importance of unspecific supportive measures to prevent complications which are not different from those caused by other viruses and can lead to multiorgan failure and death (213).

Although several metanalyses have been recently published about the autoptic reports of the last two years and all agree that the main changes can be found in the lung and that necrosis of the kidney tubulus is quite often described (212-214) the question of the cause of death (215)after infection of the nasal mucosa by the virus (216-222**)** has been clearly addressed by Hooper JD et al. (223).For death certification cause of death could be devided into :

1.primary and

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2.contributing.

Acute respiratory disease was most often cited as the primary cause as „COVID-19 pneumonia“ or „acute lung injury“ in 75% of the cases.

In 25% of the cases where COVID-19 was not the primary cause of death COVID 19 was listed das as a contributing cause in 6 cases.

Inspite of the fact that 54% of the 135 decedents acquired acute kidney disfunction and 35% acquired myocardial dysfunction,these organ disturbances were not reported as primary cause of death but mostly reported as a contributing cause of death.

There was however no explantion for the „fluid inbalances“ which likely contributed to the „congestion“ and to the 3- to 4- fold increase of the lung weight which was frequently reported on the one side and the acquired kidney injury with hallmark of the tubulus necrosis on the other .

None of the autptic studies reported about the amount of fluid found in the different organs and most importantly in the heart chambers and in the urinary bladder.

Human organs are made to 80% of water (224). Water is essential for the formation of the secretions of the salivary glands, of the stomac,of the liver and of the pancreas which allow food digestion and passage of nutrients from the intestine into the portal blood. Water is important for the secretion of the glands of the upper respiratory tract and of the bronchial mucosa which represent the main barrier against noxious agents present in the air(225).

This has to be taken into consideration when supportive measures to improve prognosis of COVID-19 patients are being considered (29,157,226-228).

Water is very important for the production of the surfactant factor in the alveoli which allows the passage of oxygen and of CO2 at the level of the pulmonary capillary(61,212).The proper hydration status of the endothelial cells is exetremely important to avoid shrinking of those cells in areas such as the lung capillary where the endothelial cell represents the barrier for the intravasal fluid and prevents passage of plasma conponents into the alveolus(29,156,171).

Of course water is of vital importance for the physiological excretory function of the kidney and for the proper function of the brain.

Thirst is the central sensation which is triggered when dehydration induces 1-2% of body mass loss (227).

This induces a wide range of extracellular (decrease of blood volume and arterial pressure) and intracellular (e.g increased plasma osmolality) changes and of stimuli.

With increasing age, dehydration can continuosly develop by the reduced sense of thirst , reduced mobility and ,again, uptake of diuretics.“Chronic“ hypertonicity of the blood due to dehydration has been described in older people.It is a risk factor for morbidity and mortality and it has to be kept in mind when approaching an older patient (29,225), especially if fever above 38 degrees centigrade aggravetes this condition when lasting several days.

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The geriatric population faces two main hydration-related conditions which can lead to the need for help from the doctor namely:

*a)*dehydration when the total body fluid is significantly reduced compared to the age-specific hydration status (which is already reduced compared to that of the young) and

b) excess of body fluid (overhydration) when the amount of fluid in the body is higher than normal.

The stability of this equilibrium decreases with increasing age and a few hundred milliliters of fluid may switch the hydration status into the one or the other direction(224-226).

Fluid loss can be caused by exposure to higher temperatures without compensation, by use of diuretics and, more importantly, by protracted high fever and inadequate fluid intake.

Oral intake of fluid for compensating fluid loss under such conditions may be inadequate and prolonged,moderate intravenous fluid administration may become quickly necessary to revert the established compensatory mechanisms to the dehydration condition before homeostasis is reestablished.

The consequence of fluid loss from the body is the reduction of blood volume, hyperconcentration with appearence of symptoms like dizzines, confusion (or delirium,brain fog), seizure and eventually stroke and death (224,225).

Dehydration in addition to reduced blood volume and eventually hypoalbuminemia may be responsible for other ominous symptoms, namely hypoxia (226) but can also predispose to diffuse thromboembolism especially in the lung (226-230).

This phenomenon may be the cause of progressive „sequestration“ of blood and of the progressive weight gain of the lung and eventually for reduction of blood volume and weight reduction in other organs especially in the kidney(181) .

Reduction of blood volume leads finally to cardiac arrest, the definitive cause of death (215) comparable to what happens in cases of malignant hyperthermia where fluid loss causes shock withincreased lung weight (231).

The Future of symptomatic HuCoV-2-infection is in Home Treatment for Hospitalisation Prevention

The best would be to prevent COVID-19-positive patients to need hospitalisation by keeping them under controlled treatment at home .Although the symptoms caused by HuCoV-2-infection of the upper respiratory tract have been very much similar to those caused by the other endemic 4 coronaviruses and by the different influenza viruses, no official recomendations for farmacological treatment were delivered by the health authorities and this was justified mostly because of lack of prospective trials.

After the first phase of the pandemic,which caused hundred of deaths of doctors who attempted to deliver relief and save lives without availability of enough preventive cloths and masks .

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general practitionaries were not willing any more to visit patients at home.

A report about the results of an online survey among the 8.000 members of the Italian College of General Practitioners and Primary Care (SIMG) performed in the period from january first 2021 and february 8 2021 was published im March 20th 2021,(22).

Members of the society were asked the daily problems with the treatment of COVID-19 patients at home.The question how to treat

patients with fever was answered with the suggestion to use up to 3 grams paracetamol per day.The use of other drugs such as aspirin or iboprufen was also recommended.

It was also recommended to monitor an appropriate state of nutrition and hydration.

Monitoring of body weight was not however recommended.The fact however that the suggestions were not brought into practice

allowed, in may cases, disease to progress to respiratory distress and weakness (most probably in those patient who continue to take anti-hypertensive drugs in spite of developement of hypotension) and forced patients and relatives to seek help in emergency rooms,infectious disease wards and eventually ICU.

In a retrospective analysis Suter and coworkers found that early treatment of COVID-19 patients at home prevented hospital admission due to progression to respiratory distress and in in over 90% reduction of numbers of hospitalisation days and treatment costs (18).

An important prospective study performed in Denmark should pave the way to act in this direction (19,20).

In that prospective study only 28 out of 378 COVID-19, symptomatic patients sought temporary support at the hospital or at a COVID assessment unit.Twelve of them belonged to the usual-care group (188 patients);their urgent care visits lasted from few hours to 6 days.In the PEP selfcare group (190 patients) urgent care visits were reported by 16 patients;the length of their visits lasted from two hours to three days.

At least in one case dehydration and not hypoxia was the cause of the visit.

This experience is of pivotal importance for different reasons:

1.the vast majority of symptomatic HuCoV-2 patients (378 of 8386 persons with positive PCR-test result) did not need hospitalisation and recovered without further treatment.

2.patients could seek help also in so called COVID-19 assessment units.

3.as no difference in respiratory scale was observed between the two groups, the secondary outcome,hospitalisation related to HuCoV-2-infection, may not be due to the direct viral effect.

4.partecipants were reccomanded to have sufficient intake of fluid especially when fever appeared and to take paracetamol not only to decrease body temperature in case of fever but also to reduce myalgia or headace (18)

3.none of the hospitalized patient died.

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4.it may not be reproducible in all parts of the world as it may need a high cultural level, as the partecipants were tested for their „understanding“ of health informations and their knowledge about what to do and how to interact with health care providers.

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