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Examine the Lung Role in Acute Respiratory Distress Syndrome and COVID-19-Disease with Focus on Transpulmonary Gradient of the Inflammatory Biomarkers

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I. List of abbreviations

A2Macro	**Alpha-2-Macroglobulin**
AAT	**Alpha-1-Antitrypsin**
ACE-2	**Angiotensin Converting Enzyme 2**
AFP	**Alpha-Fetoprotein**
ANG-1	**Angiopoeitin-1**
ARDS	**Acute Respiratory Distress Syndrome**
AXL	**AXL Receptor Tyrosine Kinase**
B2M	**Beta-2-Microglobulin**
BDNF	**Brain-derived Neurotrophic Factor**
C3	**Complement C3**
CA-125	**Cancer Antigen 125**
CA-19-9	**Cancer Antigen 19-9**
CA-9	**Carbonic Anhydrase 9**
CAP	**Community Acquired Pneumoniae**
CEA	**Carcinoembryonic Antigen**
CO_2	**Carbonic dioxide**
COVID-19	**Coronavirus Disease 19**
CRP	**C-reactive Protein**
EDTA	**Ethylenediaminetetraacetic acid**
EN-RAGE	**Extracellular Newly identified Receptor for Advanced Glycation End products binding protein**

FAS	**FAS Ligand Receptor**
F_iO_2	**Fraction of Inspired Oxygen**
FRTN	**Ferritin**
g	**Gram**
GM-CSF	**Granulocyte-Macrophage Colony Stimulating Factor**
H_2O	**Water**
HAP	**Hospital Acquired Pneumoniae**
HCC-4	**Human Chemokine CC-4**
hCG	**Human Chorionic Gonadotropin beta**
HGF	**Hepatocyte Growth Factor**
ICAM1	**Intercellular Adhesion Molecule 1**
ICU	**Intensive Care Unit**
IFN	**Interferon**
IgA	**Immunglobulin A**
IgM	**Immunglobulin M**
IL	**Interleukin**
l	**Liter**
Lp(a)	**Apolipoprotein(a)**
LPS	**Lipopolysaccharide**
MCP	**Monocyte Chemotactic Protein**
mg	**Milligram**
MIP	**Macrophage Inflammatory Protein**

μg	**Microgram**
ml	**Milliliter**
mmHg	**Millimeter of Mercury**
MMP	**Matrix-Metalloproteinases**
NAAT	**Nucleic Acid Amplification Test**
ng	**Nanogram**
NO	**Nitric Oxide**
NSE	**Neuron-Specific Enolase**
O_2	**Oxygen**
PAI-1	**Plasminogen Activator Inhibitor 1**
P_aO_2	**Partial Pressure of Oxygen**
PARC	**Pulmonary and Activation-Regulated Chemokine**
PCR	**Polymerase Chain Reaction**
PECAM-1	**Platelet Endothelial Cell Adhesion Molecule**
PEEP	**positive End-Expiratory Pressure**
pg	**Picogram**
pH	**Potential of Hydrogen**
RAGE	**Receptor for Advanced Glycation End-products**
RANTES	**Regulated on Activation, Normal T Cell Expressed and Secreted**
RNA	**Ribonucleic Acid**
ROS	**Reactive Oxygen Species**
SAPS	**Simplified Acute Physiology Score**

SAP	**Serum Amyloid P-component**
SARS-CoV-2	**Severe Acute Respiratory Syndrome Coronavirus 2**
SCF	**Stem Cell Factor**
SIRS	**Systemic Inflammatory Response Syndrome**
SP-D	**Surfactant Protein D**
TBG	**Thyroxine-Binding Globulin**
TISS	**Therapeutic Intervention Scoring System**
TNF	**Tumor Necrosis Factor**
TNFR2	**Tumor Necrosis Factor Receptor 2**
TRAIL-R3	**TNF-Related Apoptosis-Inducing Ligand Receptor 3**
VCAM-1	**Vascular Cell Adhesion Molecule-1**
VDBP	**Vitamin-D-Binding Protein**
VEGF	**Vascular Endothelial Growth Factor**
vWF	**von Willebrand Factor**
WHO	**World Health Organization**

1. Abstract

Background: The main function of the lungs is respiration, however there are a few studies about other metabolic functions of the lungs, i.e. production of the angiotensin converting enzyme and thus participate in the metabolism of angiotensin. There is also data on the metabolism of some drugs through the lungs.

100% of the cardiac output flows through the lungs. In principle, this results in the prerequisites that the lungs also intervene in metabolic processes or significantly change the blood concentration of biomarkers i.e. inflammatory mediators. We hypothesize that numerous metabolites of the blood are changed passing through the lung or the lung secrete certain inflammatory mediators into the blood.

The present work compares the transpulmonary gradient of inflammatory cytokines, mentioned as delta value through the work, among three different groups: healthy individuals, pneumonia / ARDS patients and COVID-19 patients. The delta values are defined as the difference of biomarkers concentrations found in serum taken from arterial blood (oxygenated, after passing through the lungs) and biomarkers concentration found in serum taken from the central venous blood (deoxygenated, before entering the lungs).

Methods: Men from January 2019 to April 2020 were included in our study. The samples were collected at the Saarland University Hospital (UKS). Three groups were included: patients from surgery department as control group (sample size n = 26), pneumonia / ARDS group (n = 23) and COVID-19 group (n = 10). To collect samples, blood was drawn from the central venous catheter (CVC) and peripheral arterial catheter. The statistical evaluation was carried out using the program SPSS Version 26 (IBM 2019).

Results: The age distribution ranged from 34 to 93 years old, with the mean age being 62.17 ± 12.69 years old. According to the Shapiro-Wilk test ($p > 0.05$), the age distribution of the subjects was normal.

The survival of patients was also documented. No one from control group died. From the “pneumonia / ARDS” group, 3 out of 23 patients died and from the “COVID-19” group 5 out of 10 patients died in the course of the disease.

We tested 76 different cytokines and biomarkers, in both arterial and venous circulatory system. 4 biomarkers differed significantly in their delta value between the COVID-19 and control groups, 2 biomarkers between the pneumonia / ARDS and the control groups and 3 biomarkers between the pneumonia and COVID-19 groups.

The delta values of nine biomarkers correlated significantly with the intensive care medicine scores SAPS (Simplified Acute Physiology Score) and TISS (Therapeutic Intervention Scoring System) in pneumonia / ARDS group. In contrast, a correlation between the biomarkers in COVID-19 group and the intensive medicine scores could not be detected.

The comparison between the survived and deceased COVID-19 patients revealed a significant difference in the delta values of 3 biomarkers.

Conclusions: Cytokines and biomarkers can make statements about the disease and the course of COVID-19 and pneumonia / ARDS patients. In our study the transpulmonary gradient of some pro- and anti-inflammatory biomarkers were statistically significant among the three groups including IgM, IL-1RA, IL-10 among others. The transpulmonary gradient of these biomarkers were the lowest in COVID-19 group, however comparing the venous and arterial samples within each group, COVID-19 group had the highest venous and arterial concentration of theses biomarkers. This might be an indicator for a higher inflammation state in COVID-19 in comparison to other types of ARDS. Furthermore, we could show a correlation between the transpulmonary gradient of two cellular adhesion molecules and outcome of our COVID-19 patients.

1.1. Zusammenfassung

Die Hauptfunktion der Lunge ist der Austausch der Atemgase, jedoch es gibt ein Paar Studien über die andere metabolische Funktionen der Lungen, z. B. die Produktion des Angiotensin-Converting Enzymes und somit Beteiligung am Stoffwechsel von Angiotensin. Weiterhin gibt es Daten zur Metabolisierung einiger Pharmaka durch die Lunge.

Die Lunge wird von 100 % des Herzzeitvolumens durchströmt. Dadurch ergeben sich prinzipiell die Voraussetzungen, dass die Lunge auch in metabolische Prozesse eingreift bzw. die Blutkonzentration von beispielsweise Entzündungsmediatoren deutlich verändert.

Wir hypothetisieren hier, dass zahlreiche Metabolite des Blutes im Rahmen der Lungenpassage verändert werden beziehungsweise die Lunge bestimmte Entzündungsmediatoren ins Blut abgibt.

Die vorliegende Arbeit Vergleich die transpulmonale Gradienten der Entzündungsmediatoren, der in dieser Arbeit als Delta-Wert bezeichnet wird, zwischen drei verschiedenen Gruppen ab: Gesunde, Patienten mit Pneumonie / ARDS und Patienten mit COVID-19 Erkrankung. Die Delta-Werte sind definiert als die Differenz der Biomarkerkonzentration im Serum aus arteriellem Blut (oxygeniert, nach Durchtritt durch die Lunge) und der Biomarkerkonzentration im Serum aus dem zentralvenösen Blut (desoxygeniert, bevor es in die Lunge gelangt).

Methoden: In unsere Studie wurden männliche Patienten von Januar 2019 bis April 2020 eingeschlossen. Die Proben wurden am Universitätsklinikum des Saarlandes, Deutschland gesammelt. Es wurde zwischen drei Gruppen unterschieden. Zum einen die Operationspatientengruppe als Kontrollgruppe (Stichprobengröße n = 26), die Pneumonie- / ARDS-Gruppe (n = 23) und die Gruppe mit COVID-19-Patienten (n = 10). Um die einzelnen Proben einer Person zu sammeln, wurde Blut aus dem zentralen Venenkatheter (ZVK) und dem peripheren arteriellen Katheter entnommen. Die statistische Auswertung erfolgte mit Hilfe des Programms SPSS Version 26 (IBM 2019).

Ergebnisse: Die Altersverteilung über die gesamte Studie reichte von 34 bis 93 Jahren, wobei das Durchschnittsalter 62,17 Jahre mit einer Standardabweichung von 12,69 Jahren betrug. Laut Shapiro-Wilk-Test (Signifikanz $p > 0,05$) war die Altersverteilung der Probanden normal.

Auch das Überleben der Patienten wurde dokumentiert. Es zeigte sich, dass kein Patient der „gesunden“ Gruppe starb. Aus der Gruppe „Pneumonie“ starben 3 von 23 Patienten und aus der Gruppe „COVID-19“ starben 5 von 10 Patienten im Krankheitsverlauf.

Insgesamt wurde das jeweilige Blut auf 76 verschiedene Zytokine und Biomarker, sowohl arteriell wie auch venös, getestet. Die Hauptergebnisse waren, dass sich insgesamt 4 Biomarker

in ihrem Delta-Wert zwischen den COVID-19-Patienten und Patienten mit gesunden Lungen signifikant unterschieden. Im Vergleich dazu wurde bei insgesamt 2 Biomarkern ein signifikanter Unterschied zwischen den Pneumonie- bzw. ARDS-Patienten und den gesunden Probanden gefunden. Die Pneumonie- und COVID-19-Patienten unterschieden sich hingegen in insgesamt 3 Biomarkern. Die Blutkonzentration der restlichen Biomarker unterschied sich beim Durchgang durch die Lunge nicht statistisch signifikant.

Für die intensivmedizinischen Scores SAPS und TISS konnten für die Gruppe der Pneumoniepatienten 9 korrelierte Marker bestimmt werden. Ein Zusammenhang zwischen COVID-19-Patienten und den Scores konnte hingegen nicht nachgewiesen werden.

Der Vergleich zwischen den überlebenden und verstorbenen COVID-19-Patienten ergab einen signifikanten Unterschied in den Serumkonzentrationen für 3 Biomarker.

Schlussfolgerungen: Anhand der Zytokin- und Biomarkerwerte können Aussagen über die Erkrankung und den Verlauf von COVID-19- und Pneumonie/ARDS-Patienten getroffen werden. In unserer Studie waren die transpulmonalen Gradientenunterschiede einiger pro- und antiinflammatorischer Biomarker zwischen den drei Gruppen einschließlich IgM, IL-1RA, IL-10 statistisch signifikant. Der transpulmonale Gradient dieser Biomarker war in der COVID-19-Gruppe am niedrigsten, aber beim Vergleich der venösen und arteriellen Proben innerhalb jeder Gruppe wies die COVID-19-Gruppe die höchste Konzentration dieser Biomarker in beiden Proben auf. Dies könnte ein Indikator für einen höheren Entzündungszustand bei COVID-19 im Vergleich zu anderen Arten von ARDS sein. Darüber hinaus konnten wir eine Korrelation zwischen dem transpulmonalen Gradienten zweier zellulärer Adhäsionsmoleküle und dem Outcome von COVID-19-Patienten aufzeigen.

2. Introduction

2.1. Acute Respiratory Distress Syndrome

The acute respiratory distress syndrome (ARDS), originally described in 1967, is a medical condition characterized with an acute generalized inflammation of the lungs that leads to a severe shortness of breath, rapid breathing, pulmonary edema, extreme tiredness, decreased lung elasticity, among other symptoms and signs. The need for mechanical ventilation is common [1]. In 1992, an American–European consensus conference recognized specific diagnostic criteria for the disease [2]; they were revised in 2012 in the so-called Berlin definition [3] of ARDS in adults (Table 1). Oxygenation level determines the diagnosis of ARDS, categorised as 'mild', 'moderate' and 'severe' (Table 1). The diagnosis of ARDS only depends on clinical criteria since it is not easily feasible to get precise evaluation of lung damage by taking tissue sample of the lung in most patients; moreover, neither distal airspace nor blood can be utilized to diagnose ARDS. ARDS occurs mainly in the setting of pneumonia (bacterial and viral; fungal appears in a lesser extent), non-pulmonary sepsis (peritoneum, urinary apparatus, soft tissue and skin), aspiration of gastric, oral and esophageal secretions (produced by successive infection) and major trauma (e.g., blunt or penetrating injuries or burns). The clinical course of ARDS differs depending on the geographical venue, the efficiency of health care systems and the availability of health resources; thus, the morbidity and mortality rates alter according to development level of the country.

2012 Berlin Definition	
➢ Timing:	Respiratory failure within 1 week of a known trauma or new and/or worsening respiratory symptoms
➢ Origin:	Respiratory failure not fully justified by cardiac function or volume overload (need objective criteria such as echocardiography to exclude hydrostatic edema if no risk factor is present)
➢ Imaging:	Bilateral opacities on chest radiograph and/or computed tomography not fully explained by effusion, collapse or nodules
➢ Oxygenation:	Acute onset of hypoxemia specified as PaO_2/FiO_2 < 300 mmHg on at least PEEP 5 cm H_2O [1]

	- PaO_2/FiO_2 of 201 - 300 mmHg is mild ARDS - PaO_2/FiO_2 of 101 - 200 mmHg is moderate ARDS - $PaO_2/FiO_2 \leq 100$ mmHg is severe ARDS
2016 Kigali Modification [2]	
➢ Timing and origin:	As in the Berlin definition
➢ Imaging:	Bilateral opacities on chest radiograph and/or ultrasonography not fully explained by effusion, collapse or nodules
➢ Oxygenation:	$SpO_2/FiO_2 < 315$; no PEEP requirement
[1]**PEEP** may be delivered non-invasively if the criteria are in the mild group. [2]The Kigali definition was not directly compared with the Berlin definition in the initial publication; patients in the Kigali study were not getting artificial ventilation. **ARDS**, acute respiratory distress syndrome; **FiO_2**, fraction of inspired oxygen; **PaO_2**, partial pressure of arterial oxygen; **PEEP**, positive end-expiratory pressure; **SpO_2**, peripheral oxygen saturation.	

Table 1. Definitions of ARDS in adults

The so-called LUNG-SAFE study (**L**arge observational study to **UN**derstand the **G**lobal impact of **S**evere **A**cute respiratory **F**ailur**E**) was a multicenter, prospective, observational, four-week inception cohort study where current data on epidemiology can be found [4]. In the study, in an appropriate sample size of 459 ICUs from 50 countries across 5 continents the results were as follows: out of 29144 hospitalized patients to participating ICUs, 3022 (10.4%) conformed ARDS criteria, in which 2377 had ARDS in the first 48 hours and whose respiratory failure was coped with invasive assisted ventilation. The period prevalence of mild ARDS was 30.0%; of moderate ARDS, 46.6%; and of severe ARDS, 23.4%. ARDS constituted 0.42 cases per ICU bed over 4 weeks and corresponded 10.4% of ICU entries and 23.4% of patients needing assisted ventilation.

The data from the LUNG-SAFE study were lately analyzed independently in a subgroup analysis for Germany [5]. Of the 7540 hospitalized patients to 95 ICUs from 18 university and 62 non-university hospitals in May 2004, mechanical ventilation was given to 1028 and 198 developed ARDS (19%). Although the physical characteristics of ARDS patients were

comparable, hospital mortality was considerably lower in university in comparison to non-university hospitals (39.3% vs 57.5%; $p = 0.012$). The statistics must consequently be regarded as a sign of an overall greater quality of treatment in such centers.

2.1.1. The role of biomarkers

Unfortunately, the origin of the ARDS cannot always be evidently diagnosed and remains unclear in around 8% of the cases. This is one of reasons to study and identify biomarkers in ARDS [6, 7]. Among many others, angiopoietin-2, an endothelial growth factor, and the epithelial receptor RAGE (Receptor for Advanced Glycation End products) have been found as potential biomarkers [8, 9]. Because RAGE is highly expressed on alveolar epithelial cells, its plasma concentration mainly reflects epithelial injury. Thus, it seems to be appropriate as a marker for the extent of ventilator-associated lung injury [10]. Contrarily, plasma concentration of angiopoietin-2 is mostly an expression of endothelial damage.

➢ Epithelial markers (principal source)	- Receptor for advanced glycation end products (alveolar epithelial type 1 cells) - Surfactant protein D (alveolar epithelial type 2 cells) - Club cell 16 (airway epithelial cells)
➢ Endothelial markers (principal source)	- von Willebrand factor (endothelium and platelets) - Angiopoietin 2 (endothelium and platelets) - Intercellular adhesion molecule 1 (endothelium, epithelium and macrophages) - Syndecan (endothelial glycocalyx) - Endocan (endothelium)
➢ Inflammatory markers (principal source)	- IL-6 (monocytes, macrophages, neutrophils and alveolar epithelium) - IL-8 (monocytes, macrophages, endothelium and alveolar epithelium) - Soluble tumour necrosis factor receptor 1 (alveolar epithelial

	type 1 and type 2 cells and macrophages) - IL-1β, IL-1R antagonist (monocytes, macrophages and alveolar epithelium) - Neutrophil extracellular traps (neutrophils)
➢ Coagulation and fibrinolysis markers (principal source)	- Protein C (plasma) - Plasminogen activator inhibitor 1 (endothelium and macrophages)
➢ Apoptosis markers (principal source)	- FAS and FasL (endothelium, alveolar endothelium and inflammatory cells)
Selected based on several clinical studies that were focused on the pathogenesis and prognosis of ARDS. **ARDS**, acute respiratory distress syndrome; **FAS**, tumour necrosis factor receptor superfamily member 6; **FasL**, tumour necrosis factor ligand superfamily member 6.	

Table 2. Selected biomarkers associated with human ARDS

Several of these biomarkers have also been related to poor clinical outcomes in ARDS (Table 2), indicating that the extent of the lung endothelial and epithelial damage in humans is a determining factor of clinical outcome [11].

2.2. Pneumonia

Community-acquired pneumonia is still the most common infectious disease leading to hospitalization and remains associated with considerable morbidity and mortality. despite of the quick progress of new treatments, pneumonia is still the reason for a high rate of health complications, sepsis, septic shock and death worldwide [12, 13, 14]. It is classified as either CAP (community-acquired pneumonia) or nosocomial, based on the environment from which the patient caught the disease.

Almost all cases of pneumonia come from bacterial infection, which is normally cured with antibiotics. Nevertheless, some noninfectious sources, such as pulmonary embolism, malignancy, and congestive heart failure may also result in symptoms similar to CAP. Besides, viral pneumonia is a well-defined entity, especially among immune-compromised patients [15]. In such situations, the inaccurate diagnosis is generally thought only after failure of the

antibiotic therapy, and the life-threatening risks related with these untreated nonbacterial causes rise quickly [16,17].

In the literature, the incidence rate of therapy failure in CAP is mentioned 6% to 31% [18, 19, 20, 21, 22], with 5-13% of all patients with hospitalised CAP getting progressive pneumonia [18, 23, 24]. After achieving clinical stability, the rate decreases to nearly 1% [25]. The mortality rate is exceptionally elevated even if treatment fails owing to secondary, nosocomial acquired pneumonia [26, 27].

Nosocomial pneumonia is normally separated into two different groups: hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) [28].

The largest data on the epidemiology of HAP proceed from the databases of the surveillance systems of nosocomial infections. In Germany, the Infection Surveillance System (in german, Krankenhaus-Infektions-Surveillance-System, KISS) delivers most of the data on this subject matter.

There are about 11,300 VAP cases per year in the intensive care units (ICU) in Germany [29]. Corresponding to the data from the 2011 national prevalence study, 18.7% of the total nosocomial infections are pneumonia [30, 31].

Cassini et al. have published an extrapolation of the effects of nosocomial infections based on the point prevalence studies in the EU countries 2011/2012 [32]. As stated in this, an incidence of 138 HAP per 100,000 inhabitants is estimated, corresponding to almost 113,000 HAP per year in Germany, given that the incidence of HAP in Germany nearly experiences the European average.

VAP is a potentially fatal complication in the ICU and is correlated with longer time of mechanical ventilation, extended hospital stay, boosted treatment costs, and enhanced attributable mortality [33]. One of the most demanding problems in VAP is the absence of a “gold standard” procedure of diagnosis [34]. The normally used criteria are based on clinical factors that lack specificity [35].

2.2.1. The role of biomarkers in pneumonia

Searching for the ideal biomarker for pneumonia is in progress, and several molecules are undergoing thorough studies [36]. C-reactive protein (CRP) and procalcitonin (PCT) continue to be the broadly used biomarkers, while interleukin 6 (IL-6) has been of special importance research-wise [37–40]. Serious inflammatory processes cause many changes in cellular metabolism [41]. Neugebauer et al. described various metabolic patterns (metabolome) specific for sepsis and CAP; additionally, they demonstrated that Putrescine (a polyamine) is a predictor

for CAP [42]. A different study proposed that metabolomics is able to differentiate CAP from other noninfective pulmonary acute conditions with high specificity and sensitivity, and that specific metabolites can substantially distinguish fatal from nonfatal CAP cases, thus being indicators of survival [43].

2.3. COVID-19

A novel viral pneumonia known as coronavirus disease 2019 (COVID-19) diagnosed first in Wuhan, China in December 2019. The World Health Organization (WHO) subsequently announced COVID-19 as a pandemic in March 2020 [44]. COVID-19 is caused by a novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronaviruses are positive, single-stranded RNA (ssRNA) viruses that lead to serious illness in mammals and birds, predominantly respiratory and intestinal infections [45].

The common symptoms of this disease are cough, fever, and dyspnea, and it can progress to pulmonary failure and cause liver, heart, and kidney injuries. Clinical management of critical cases entails respiratory assistance [46].

Angiotensin converting enzyme-2 (ACE2) regulates SARS-CoV-2 (as well as other coronaviruses) access via clathrin-mediated endocytosis into type-2 pneumocytes and macrophages of the lung tissue [47,48]. ACE2 is a membrane-bound enzyme that transforms active angiotensin II to inactive angiotensin. Consequently, ACE2 stops the effects of angiotensin II action, allowing for classic vasoconstriction in addition to inflammation and thrombosis. The loss of ACE2 function following binding by SARS-CoV-2 expands in situ pulmonary inflammation and coagulation as unfavorable effects of declining counter regulation of the angiotensin II/AT1 receptor axis [49].

The infection can produce endotheliitis and hypercoagulability and therefore thromboembolism not only in patients with pre-existing health conditions, but also in healthy people and can cause multiple organ failure [50-54].

The mortality rate from COVID-19 was over 10% in some regions. Perhaps considering the fact that the health system was overloaded, played a role here.

6294 new cases per day was the highest number in Germany at the peak of the pandemic on March 27th, 2020. In the first week of June, there were 214 to 507 new cases per day and grew again by the end of July 2020 to 305 to 1012 cases per day. By July 31st, 2020, a total of 209653 cases emerged in Germany and 9148 deaths were reported. Worldwide, as of July 31st,

2020, approximately 17,6 Mio. SARS-CoV-2 infected people and nearly 680,000 deaths were registered [55].

Severity grade of COVID-19 (defined by WHO severity scores, graded as mild, moderate, severe, or critical) is typically depending on the activation of immune response [56]. Among others, the following have been observed: Lymphocytopenia, neutrophilia and increased serum levels of IL 1β, IL-2, IL-4, IL-6, IL-10, TNF-α, and Interferon-γ. This cytokine response is linked with cell damage, which causes in elevated serum levels i.e., of lactate dehydrogenase (LDH), heart and liver enzymes, in addition to activation of coagulation and fibrinolysis with considerably elevated plasma levels of D-dimer [57].

Various studies and meta-analyzes have recognized a so-called “cytokine storm” (also known as hypercytokinemia) in COVID-19 patients, as an uncontrolled and abnormal immune response in COVID-19 disease, like in severe influenza [58-64]. Above all, TNF-alpha, IL-6, IL-12, IL-8, and ferritin are vastly released throughout the course of the disease and consequently causing ARDS and multiple organ dysfunction [64].

Many studies suggest that a “cytokine storm” not only plays a role in COVID-19 pathophysiology but also has prognostic importance to monitor the clinical course of the disease [65-69].

2.4. Biomarkers

A large variety of cells are able to release cytokines that could be used as biomarkers. They predominantly control locally acting factors, but are also implicated in inflammatory processes, in the immune response as well as in defense processes. Interleukins (IL) play a key role in the inflammatory response. A distinctive feature is made between pro-inflammatory, anti-inflammatory, immune-modulatory and chemotactic interleukins, all of which have a great influence on the inflammatory reactions [70].

A biomarker, on the other hand, refers to any molecule, structure, or process that can be determined in the body or its excretions and affects and/or predicts the incidence of a disease [71]. Biomarkers are seen as a valuable way of monitoring a patient’s response to infection by anticipating the disease severity and reaction to therapy [72]. This also permits earlier and better detection of patients with critical life-threatening infections and helps the selection of a more suitable treatment method [73].

In our study, a total of 76 cytokines/biomarkers were examined in three study groups: healthy, pneumonia patients and COVID-19 patients. The complete list of the tested biomarkers and their function are in Table 19 in the appendix.

In the following text, the terms cytokines, biomarkers, and metabolites are equated.

2.5. Metabolic and secretory functions of the lung

In the pulmonary capillaries, carbon dioxide is exchanged for oxygen from the alveoli, and many compounds are produced that break down blood clots. Pulmonary endothelium is a valuable supplier of fibrinolysin activator, which transforms plasminogen present in plasma to fibrinolysin, which consequently breaks down fibrin-to-fibrin degradation products. Hence, the lung has an effective fibrinolytic system, which lyses clots in the pulmonary circulation [75]. Moreover, the lung is the richest provider of heparin (which inhibits coagulation) and thromboplastin (which by converting prothrombin to thrombin, stimulates coagulation) [75]. Thus, the lung may play a role in the whole coagulability of blood to support or suspend coagulation and fibrinolysis.

Angiotensin-converting enzyme (ACE) is present in plasma and systemic vascular endothelium but appears in much higher quantities on the endothelium of pulmonary vessels. The inactive decapeptide angiotensin I is converted into the vasoactive octapeptide angiotensin II by ACE when passing through the lung. Even though the circulation time in the interior of the pulmonary capillaries is ,1 s, 70% of the angiotensin I arriving the lung is transformed to angiotensin II [74]. Likewise, the vasoactive nonapeptide bradykinin is broken down by ACE in the lung as well. Atrial natriuretic peptide and endothelin are also eliminated by the lung.

The lung is an essential extra-hepatic location for mixed function oxidation by the cytochrome P450 system but in contrast to hepatocytes, their action cannot be stimulated. Their metabolic capability is small and easily oversaturated. In addition, a very important role of lungs may be working as a buffer by binding to intravenous drugs, stopping an acute rise in their systemic concentrations. Pulmonary extraction means the transport of a drug from the blood into the lung. Subsequently, the drug can be metabolized or moved back unaffected into the blood. Several drugs, such as anesthetic medications, are taken up, metabolized, or released gradually from the lungs [76].

The aim of this project is to investigate how the lungs intervene in the serum concentration of certain biomarkers in different disease settings and to see if lungs secrete inflammatory cytokines in the circulatory system. For this purpose, we took blood samples immediately

before and after the pulmonary circulation. In this context, “in front of the lungs” means that the blood sample is taken from the central venous catheter (CVC). This is located in the vena cava from which the blood flows to the heart and then to the lungs. “After the lungs” means that the blood sample is taken after passing through the lungs (from an arterial cannula in radial artery). These samples are then examined on inflammatory mediators to analyze how the lungs modulate blood inflammatory composition.

2.6. Aim of the work

Cytokines and biomarkers are important factors in the diagnosis and the course of the diseases [27]. Biomarkers are valuable parameter for monitoring a patient’s response to infection by considering the disease severity and reaction to therapy [31]. Likewise, this enables earlier and better detection of patients with serious life-threatening infections and helps the selection of a more suitable treatment method [16]. Thus, cytokines as part of systemic inflammation and also have an enormous influence on the course of pneumonia or COVID-19 disease. Many studies suggest that a “cytokine storm” not only plays a role in COVID-19 pathophysiology but also has a prognostic importance to predict the course of the disease [65-69].

The aim of this project is to investigate how the lung intervenes in the serum concentration of certain biomarkers and to characterize if the lungs secrete inflammatory cytokines into the circulation system.

The comparison of the results of the two samples - central venous and arterial - provides information about metabolic functions of the lungs and the role of the lungs in the elimination or secretion of inflammatory mediators. This approach opens completely new possibilities for understanding the lungs in organ related as well as systemic diseases.

Our study envisaged several goals based on these findings:

1. Is there a significant difference among the serum concentrations of the biomarkers before the lung passage and afterwards in healthy individuals, pneumonia / ARDS and COVID-19 groups?

2. Is there a correlation between the TISSSAPS intensive scores and the delta value of biomarkers concentrations in pneumonia / ARDS and COVID-19 groups?

3. Is there any correlation between the outcome of the COVID-19 group and the delta values of the biomarkers? On the other hand, which biomarkers correlate with the risk for death?

3. Materials and methods

3.1. Patient groups

After written consent, men from January 2019 to April 2020 were included in our study. The samples were collected at the Saarland University Hospital (UKS).

Only men were included in the study, as women are known to have strong metabolic fluctuations due to their menstrual cycle [77].

We divided the patients into three groups: The patient from surgery department as the control group (n = 26), the pneumonia / ARDS group (n = 23) and the COVID-19 group (n = 10).

The study was approved by the ethics committee of the Ärztekammer des Saarlandes (approval number 132/18). We obtained a written consent from the test subjects or a legal representative after a detailed medical information conversation with them.

The test subjects had as inclusion criteria (1) a medical diagnosis of pneumonia / ARDS or a test confirmed COVID-19 disease (2) as well as medical care with a central venous catheter (CVC) and a peripheral arterial Catheter.

In the control group, the criteria were (1) an extrapulmonary surgery and (2) the medical care with a central venous catheter (CVC) and an arterial cannula. An existing lung disease should not be present. In addition, the patients in the control group should not have consumed any nicotine in the past 5 years.

3.2. Diagnosis of COVID-19

To diagnose a COVID-19 disease, test material was taken from the upper or lower respiratory tract. The upper airways were tested with a nasopharynx or an oropharynx swab. Material is obtained from the lower airways by means of bronchoalveolar lavage, sputum or tracheal secretion [78].

The laboratory diagnostic of SARS-CoV-2 confirmed by a PCR test (polymerase chain reaction) [78]. This is based on the detection of unique sequences of the corona virus [79]. The nucleic acid amplification test (NAAT), which also includes reverse transcription polymerase (rRT-PCR), targets SARS-CoV-2 typical sequences, such as the genes N, E, S and RdRP [79].

3.3. Diagnosis of pneumonia and ARDS

According to guidelines, the diagnosis of community-acquired pneumonia is confirmed by a chest x-ray [12].

In nosocomial pneumonia, the diagnosis is confirmed by 1 major and 2 minor criteria (Johnson criteria) as shown in Table 3, according to the guidelines [80].

Major Criteria	New or progressive infiltrate in conventional X-ray in two planes
Minor Criteria	- Fever ≥ 38.5 ° C - Leucocytosis > 10,000 / µl or leukopenia <4,000 / µl - Purulent secretion

Table 3. The criteria of nosocomial pneumonia

Major and minor criteria for diagnosing nosocomial pneumonia.

The ARDS is defined by the internationally valid “Berlin Definition” from 2012 and shown in the table 1 [3].

3.4. Patient information

The data was completely anonymized.

The patient's date of birth, the admitting diagnosis, other diagnoses and outcome were included. In addition, the TIS and SAP scores, which were determined in the intensive care unit, were extracted from system and included in our study. The data was taken from the clinic's internal SAP system.

SAPS means “simplified acute physiology score” and TISS “therapeutic intervention scoring system”. SAPS was developed to assess the physiological status within a clinical study. For evaluation the severity of the disease, the SAPS is combined with the TISS. These scores are collected as expenditure points every 24 hours. The worst values are recorded within the past 24 hours [81, 82].

The SAPS and TISS values of our study were recorded on the day the blood was drawn.

The following tables show the calculations for the two intensive care medicine scores SAPS and TISS according to the German Institute for Medical Documentation. Tables 4, 5 and 6are intended for calculating the SAPS score, while Table 7 the TISS score [81].

	Points													
Variables	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Heart Rate (bpm)	70-119		40-69		120-159			≥160				<40		
Systolic Blood Pressure (mm Hg)	100-199		≥ 200			70-99								<70
Temperature (°C)	<39			≥39										
P_aO_2/F_iO_2 (mm Hg)							≥200			100-<200		<100		
Urine Output (L/d)	≥1,0				0,5-<1,0							<0,5		
Blood Urea Nitrogen (g/L)	<0,6													
White Blood Cell ($10^3/mm^3$)	1,0-<20			≥20										
Potassium (mmol/L)	3,0-<5,0			≥5,0 <3,0										
Sodium (mmol/L)	125-<145	≥145				< 125								
Bicarbonate (mmol/L)	≥20			15-<20			<15							
Bilirubin (µmol/L)	<68,4				68,4-<102,6				≥102,6					

Table 4. Table for determining the SAPS

The table above gives the information for calculating the SAP score.

min: minute, **mm Hg:** millimeter of mercury, **° C:** degree Celsius, **PaO2:** partial pressure of arterial oxygen, **FiO2:** fraction of inspired oxygen, **L:** liter, **d:** day, **g:** gram, **mm^3:** cubic millimeter, **mmol:** milimol, **µmol:** micromole

Points						
Variables	0	6	8	9	10	17
Chronic disease				neoplasia with metastasis	haematological neoplasia	AIDS*
Admission status **	Planned surgical	Medical	Not planned surgical			
*	Scoring in the case of a positive HIV test and corresponding clinical complications					
**	Planned surgical: The operation date is planned at least 24 hours in advance. Not planned: Surgery appointment only planned in the last 24 hours. Medical: no surgery for at least a week.					
CAVE: In the case of chronic diseases, only the one with the highest number of points may be calculated.						

Table 5. Table for determining the SAP score

The table below contains the information for calculating the SAP score.

AIDS: Acquired Immune Deficiency Syndrome, **HIV:** Human Immunodeficiency Virus

Points									
Variables	0	5	7	12	13	15	16	18	26
Age	< 40		40-59	60-69		70-74	75-79	≥80	

Table 6. Table for determining the SAP score

Category	Points per day
Mechanical ventilation	5
Multiple vasoactive medications (>1)	4
Intravenous replacement of large fluid losses (>5L/24h)	4
Peripheral arterial catheter	5
Pulmonary artery catheter	8
Hemofiltration/ dialysis	3
Measurement of intracranial pressure	4
Treatment of complicated metabolic acidosis/alkalosis	4
Single specific interventions in the ICU (naso or orotracheal intubation, cardioversion, pacemaker implantation, endoscopies, emergency surgery in the past 24 hours)	5
Specific interventions outside of ICU (surgery or diagnostic procedures)	5

Table 7. Table for calculating the TISS-28

Only the 10 most complex features are recorded in the TISS-28.

L: liters, **h:** hours, **ICU:** Intensive Care Unit.

3.5. Data collection

To collect the individual samples from a subject, we took blood from the central venous catheter (CVC) and peripheral arterial Catheter.

Approximately 20 ml blood was taken. 2 EDTA (ethylenediaminetetraacetic acid) tube with 5 ml, 2 serum tube with 10 ml and 2 RNA-PAX gene® with 5 ml.

The CVC was most often placed in the internal jugular vein. To take the blood, the distal lumen of the CVC was first closed with a clamp and then the closure cap was removed. After attaching a NaCl syringe with a content of about 10 ml, the clamp was released again and first rinsed with normal saline, then about 5 ml of blood was aspirated to prevent sample adulteration [102]. The lumen was clamped off again and the adapter for the blood tubes was connected. Then the clamp was released again, and the blood tubes were connected. The negative pressure in the three tubes filled them up. The lumen was then clamped off again and another NaCl syringe with approximately 10 ml was used after removing the adapter to rinse the lumen once more, after which it was clamped off again and got closed with a new cap. Before and after blood draw the lumen had to be disinfected [83].

The peripheral arterial catheter is usually placed in the radial artery. To take blood, the closure cap was removed first and after attaching a 2.5 ml syringe and opening the patient's side of the peripheral arterial catheter, about 2.5 ml of blood was aspirated to prevent sample adulteration. The patient's side was closed again and the adapter for the blood tubes was connected. Then the patient's side of the catheter was released again and the blood tubes were attached to the adapter. The negative pressure in the three tubes filled them up. The patient's side was then closed again, and the tubes were removed, and the lumen was washed with NaCl and a new closure cap was used, afterwards the patient's side was opened again and was washed with NaCl till the system seemed clear again.

We then centrifuged the samples from the EDTA and serum tubes in the Beckmann Coulter type Allegra X-30R Centrifuge.

The EDTA samples were centrifuged at 2500×g for 20 minutes at 20°C. The serum tubes, had to be allowed to clot for 30 minutes before centrifugation and the samples could then be centrifuged at 1300×g for 10 minutes at 20°C.

EDTA and serum were pipetted off immediately after centrifugation. In the case of the EDTA samples, the supernatant and the serum were frozen at -80°C and stored. Only the supernatant of the serum samples was frozen.

The subsequent sample transport to the biomaterial bank took place on dry ice.

3.6. Measurements of the blood concentrations of the biomarkers

The samples were evaluated using the multiplex cytokine array from Myriad (City, USA). A multiplex is an assay in which several biomarkers are quantified simultaneously in one run [84].

Until the test was carried out, all samples were stored at less than -70°C [85].

The procedure was based on the prescribed protocol and the individual cytokines. A part (aliquot) of each sample was added to individual multiplexes of the selected MAP (multi-analyte profile) and to a blocker. Different assays were used for the different cytokines [85].

The Human Inflammation MAP® v 1.1 was used for the inflammatory biomarker and was therefore used for the evaluation of most of the cytokines in our study [86].

In addition, special Custom Maps® were created in order to test individual biomarkers that would otherwise be distributed over several multiplexes [84]. These included the cytokines AXL, HCC-4, FAS, HGF, TRAIL-R3, AFP, CA-125, CA-19-9, CEA, hCG, NSE, MMP-1, MMP-7, MMP-9 total, ANG -1, CA-9, Decorin, IL-18bp, PECAM-1 and SP-D.

3.7. Statistical methods

We did the statistical analyze with the program SPSS Version 26 (IBM 2019). All biomarkers were examined for their significance regarding delta value (the concentration of biomarker in the peripheral arterial catheter minus its concentration in CVC). A significance value of $p<0.05$ applied to all tests.

The tables and self-designed figures were created with the help of the programs SPSS, Excel and Word.

The comparison among the three groups (healthy, pneumonia / ARDS and COVID-19) was determined using the Kruskal-Wallis test.

Box plots were used to show the distribution of the cytokines. The box itself represents the 1st and 3rd quartile. The 1st quartile corresponds to the value below which 25% of the values lie, and the 3rd quartile corresponds to the value below which 75% of all values lie. The line in the box plot itself represents the median [63]. The circles in the graphics represent outliers that are at least one and a half times the box length away from this. The extreme outliers, on the other hand, are marked by the asterisk (*). These are outliers that are up to three times the box length away from the 1st and 3rd quartiles [63].

To compare the venous and arterial samples, paired t-test was applied. Error bars were used to show the distribution of some of these biomarkers.

The correlation between the intensive care medicine scores SAPS / TISS and the delta value of the biomarkers was evaluated by the Spearman or Pearson test. First, the file had to be split into

the three different diagnosis groups (healthy, pneumonia / ARDS, COVID-19). The data were examined for normal distribution using the Shapiro-Wilk test. The setting “pairwise exclusion of cases” had to be considered in order not to include missing values and thus to falsify the results. The Pearson test was used for the samples with normal distribution and for the samples without normal distribution the Spearman test was used to demonstrate a positive or negative correlation between the intensive medicine scores and the delta value of biomarkers.

To analyze the relevant biomarkers in the outcome of COVID-19 and pneumonia/ARDS patients, the file was split into the two diagnosis groups and then tested for normal distribution using the Shapiro-Wilk test. Here, the “pairwise exclusion of cases” had to be considered. If the distribution was normal, the t-test was used. In comparison, the Mann-Whitney U-test was used in the absence of a normal distribution. Grouped Error bars were used to demonstrate these biomarkers. The circles of the grouped error bars represent the sample mean values [87]. The vertical lines, each bordered by a crossbar at the top and bottom, represent the 95% confidence intervals for the mean value [87].

4. Results

4.1. General patient characteristics

A total of 59 patients were included within the observation period. 26 subjects belonged to the “Healthy” group, which corresponds to 44.0%, 23 patients (38.9%) belonged to the "Pneumonia / ARDS" group and the remaining 10 subjects (16.9%) belonged to the "COVID-19" group (Figure 1).

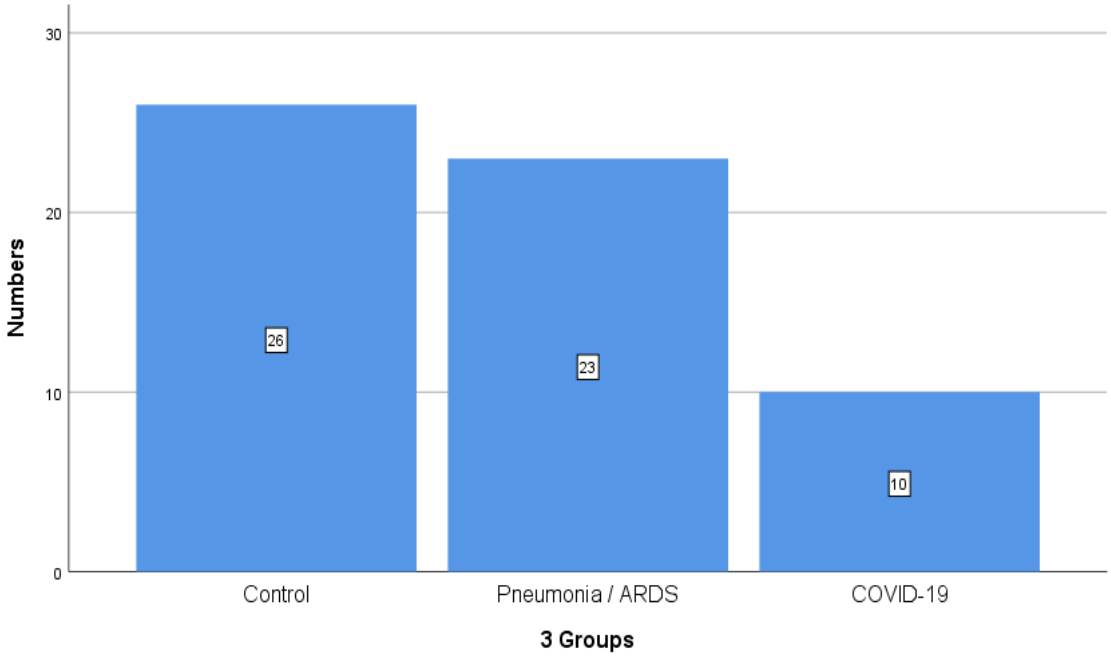

Figure 1. Number of patients in the different groups.

All patients were male. The age distribution ranged from 34 to 93 years old, with the mean age being 62.17 ± 12.69 years old. According to the Shapiro-Wilk test ($p > 0.05$), the age distribution of the subjects was normal.

The survival of patients was also documented (Table 8). No one from the “control” group died. From the “pneumonia” group, 3 out of 23 patients and from the “COVID-19” group 5 out of 10 patients died in the course of the disease.

Deceased						
3 Groups			Frequency	Percent [%]	Valid Percent [%]	Cumulative Percent [%]
Healthy	Valid	No	26	100.0	100.0	100.0
Pneumonia	Valid	Yes	3	13.0	13.0	13.0
		No	20	87.0	87.0	100.0
		Total	23	100.0	100.0	
COVID-19	Valid	Yes	5	50.0	50.0	50.0
		No	5	50.0	50.0	100,0
		Total	10	100.0	100.0	

Table 8. Deceased patients

4.2. Tested cytokines and biomarkers

In total, we tested 76 different biomarkers, both in arterial and venous samples. Table 18 in appendix shows a list of all delta values of biomarkers - arterial Concentration of biomarkers minus venous concentration-, in three different groups (control, pneumonia / ARDS and COVID-19). This table shows mean, the standard deviation, the median and the range in which the biomarkers fluctuate.

4.3. Comparison of the groups

The comparison of the three groups was carried out in intention to find a significant difference among the groups regarding the delta values of biomarkers.

We applied the Kruskal-Wallis test to show these differences. The significance value here was $p < 0.05$.

In the tables below, only those biomarkers are listed that showed a statistically significant difference among groups. The means of the delta values of the control, the pneumonia / ARDS and the COVID-19 groups, as well as the effect size are listed below. The effect size is

determined to assess the significance of a result. The effect size is defined as $r = |z / (\sqrt{n})|$. The z corresponds to the standard test statistic determined by the Kruskal-Wallis test and n corresponds to the sample size of this test. The classification according to Cohen (1992) is used to assess the size of the effect:

r: 0.10 – 0.3 corresponds to a weak effect

r: 0.3 – 0.5 corresponds to a medium effect

r > 0.5 corresponds to a strong effect

Table 20 in Appendix shows all statistically significant biomarkers regarding their delta values among three different groups with test statistic, standard deviation, standard test statistic, significance and adjusted significance. Following these biomarkers are in three separate tables respectively between two different groups listed.

Biomarkers	**Sig.**	**Mean Control**	**Mean COVID-19**	**Effect Size**
NSE in ng/mL	0.023	35.76	16.80	0.08
IgM in mg/mL	0.010	31.19	15.72	0.085
EN-RAGE in ng/mL	0.001	36.85	15.55	0.09
IL-1RA in pg/mL	0.002	35.76	16.80	0.088

Table 9. Significance values and means in control and COVID-19 groups

Biomarkers for which a significant difference could be detected, the mean from the control and COVID-19 groups, and the effect size. **L:** liter, **mL:** milliliter, **g:** gram, **mg:** milligram, **ug:** microgram, **ng:** nanogram, **pg:** picogram

Four biomarkers differed statistically significant between the control and the COVID-19 groups. The means of all biomarkers, except for MMP-7, hCG, FAS, HCC-4, CA-125, VDBPAFP, RANTES, TIMP-1, MMP-9, IL-17, IL-12p40, A2-Macro, Lp(a), BDNF, Eotaxin-1 were significantly lower in COVID-19 than in control group.

Biomarkers	Adj. Sig.	Mean Pneumonia / ARDS	Mean Control	Effect Size
PAI-1 in ng/mL	0.011	36.39	24.24	-0.05
IL-1RA in pg/mL	0.032	25.43	35.76	0.046

Table 10. Significance values and mean of control and pneumonia / ARDS groups.

Relevant biomarkers. The significance values, the mean of the control and pneumonia / ARDS groups are listed, as well as the effect size. **L:** liter, **mL:** milliliter, **g:** gram, **mg:** milligram, **ug:** microgram, **ng:** nanogram, **pg:** picogram.

Biomarkers	Sig.	Mean Pneumonia / ARDS	Mean COVID-19	Effect Size
PAI-1 in ng/mL	0.009	36.39	20.15	0.079
IL-10 in pg/mL	0.024	24.25	14.35	0.080
EN-RAGE in ng/mL	0.046	28.54	15.55	0.060

Table 11. Significance values and means for COVID-19 and pneumonia / ARDS groups.

Relevant biomarkers. These differ between the COVID-19 and pneumonia / ARDS groups. The significance values, the means for the COVID-19 and pneumonia / ARDS groups, and the effect sizes are listed for these metabolites. **L:** liter, **mL:** milliliter, **g:** gram, **mg**: milligram, **ug:** microgram, **ng:** nanogram, **pg:** picogram.

Four biomarkers differed significantly in their delta values between the COVID-19 and control groups, 2 Biomarkers between the pneumonia / ARDS and the control groups and 3 Biomarkers between the pneumonia / ARDS and COVID-19 groups.

Furthermore, we applied a paired t-test on the above-mentioned biomarkers, comparing the arterial and venous samples in each group separately. The significance value here was $p < 0.05$.

In the tables below, only those biomarkers that showed significant differences between the venous and arterial concentrations are listed. The means of biomarkers concentration taken from

venous and arterial blood, as well as the standard deviations and significances are also listed. As expected, within the control group no significant differences were seen.

Biomarkers	Venous mean (Std. Dev.)	Arterial mean (Std. Dev.)	Sig. (2-tailed)
PAI-1 in ng/mL	253.40 (125.75)	279.90 (147.85)	0.041
IL-1RA in pg/mL	599.91 (826.68)	563.43 (757.56)	0.037
EN-RAGE in ng/mL	950.56 (735.46)	827.30 (692.44)	0.021

Table 12. Significance values and means of venous and arterial samples in the pneumonia / ARDS group.

Relevant biomarkers. These differ in the pneumonia /ARDS group. The significance values, the venous and arterial means, and the standard deviations are listed for these metabolites. **L:** liter, **mL:** milliliter, **g:** gram, **mg:** milligram, **ug:** microgram, **ng:** nanogram, **pg:** picogram.

Biomarkers	Venous mean (Std. Dev.)	Arterial mean (Std. Dev.)	Sig. (2-tailed)
IL-1-beta in pg/ml	10.79 (3.98)	9.24 (3.17)	0.045
IL-1RA in pg/mL	1115.30 (641.05)	1009.0 (617.27)	0.023
EN-RAGE in ng/ml	1723.10 (787.07)	1253.90 (583.67)	0.015
Factor VII in mg/ml	290.20 (96.83)	273.90 (86.78)	0.039
IgA in mg/ml	4.25 (2.64)	3.76 (2.18)	0.029
IgM in mg/ml	4.05 (2.20)	3.70 (2.24)	0.031
TBG in mg/ml	46.50 (15.22)	41.30 (12.18)	0.022

Table 13. Significance values and means of venous and arterial samples in the COVID-19 group.

Relevant biomarkers. These differ in the group of COVID-19 patients. The significance values, the venous and arterial means, and the standard deviations are listed for these metabolites. **L:** liter, **mL:** milliliter, **g:** gram, **mg:** milligram, **ug:** microgram, **ng:** nanogram, **pg:** picogram.

To be more specific we tested the biomarkers, which differed with their delta values statistically significant among our groups, applying a paired t-test on venous and arterial samples. We will explain below, how these results related regarding the means of delta, venous and arterial values.

In our trial the delta values of EN-RAGE were statistically different between the COVID-19 and control groups as well as between COVID-19 and pneumonia / ARDS groups, but not between control and pneumonia / ARDS groups. The COVID-19 group had the lowest mean of the delta value, but the means of both venous and arterial EN-RAGE samples were the highest in this group followed by pneumonia / ARDS group. When analyzed both the venous and arterial means of EN-RAGE with paired t-test, we found out that the arterial means of EN-RAGE were lower in both COVID-19 and pneumonia / ARDS groups in comparison to venous means (figure 6).

The means of both arterial and venous IL-1RA concentrations were the highest in COVID-19 group and the lowest in the control group. The paired t-test showed statistically significant differences between the arterial and venous means of IL-1RA in pneumonia / ARDS and COVID-19 groups with lower means in arterial samples in both groups (figure 8).

The means of both venous and arterial IL-10 were higher in COVID-19 group than in pneumonia / ARDS group. Within the COVID-19 group, we found a lower arterial mean than venous mean of this biomarker. Contrarily, within the pneumonia / ARDS group, the venous mean was lower.

Regarding the PAI-1, there were significant differences of delta values between the control and pneumonia / ARDS groups as well as between the COVID-19 and pneumonia / ARDS groups. However, the means of both venous and arterial samples were higher in COVID-19 in comparison to pneumonia / ARDS group (figure 10).

The delta value of IgM in COVID-19 group was statistically lower than the control group. However, the means of both venous and arterial IgM were higher in COVID-19 group in comparison to the other groups. In paired t-test the only significant difference was seen in the COVID-19 group (figure 4).

In the following figures 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12, the relevant biomarkers are shown graphically. This corresponds to the biomarkers NSE, IgM, EN-RAGE, IL-1RA, PAI-1 and IL-10. Figures 2, 3, 5, 7, 9 and 11 serve to visualize the differences of delta values among the three groups and the figures 4, 6, 8, 10 and 12 show the same biomarkers in venous and arterial samples in all three groups.

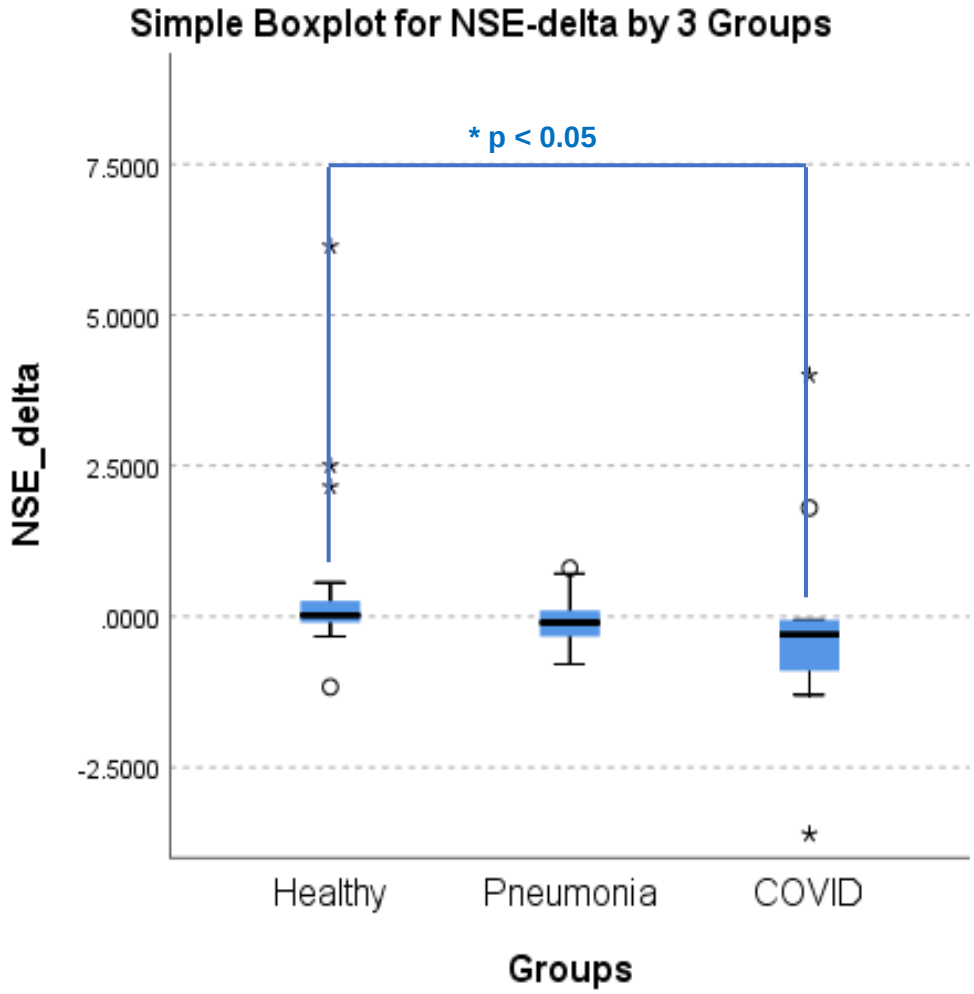

Figure 2. NSE-delta box plot comparing the three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of box plots and presentation of the medians, the quartiles and the outliers.

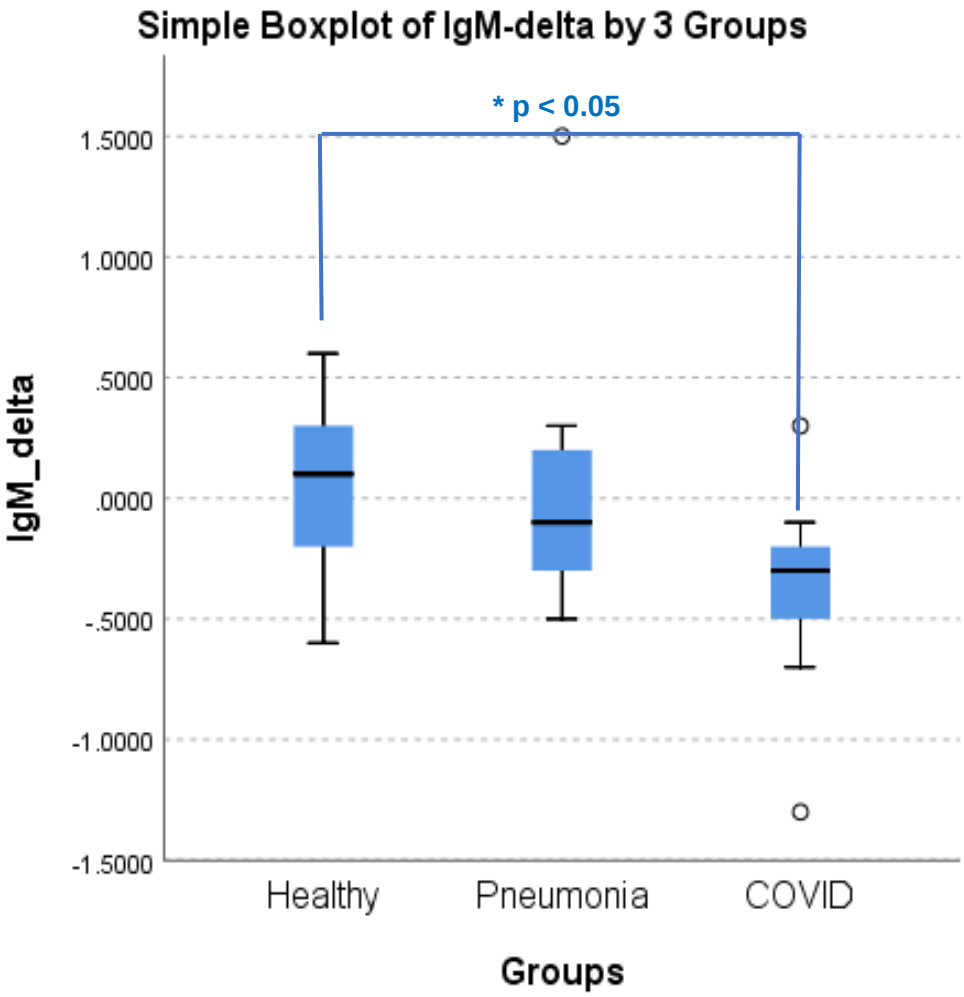

Figure 3. IgM-delta box plot comparing the three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of box plots and presentation of the medians, the quartiles and the outliers.

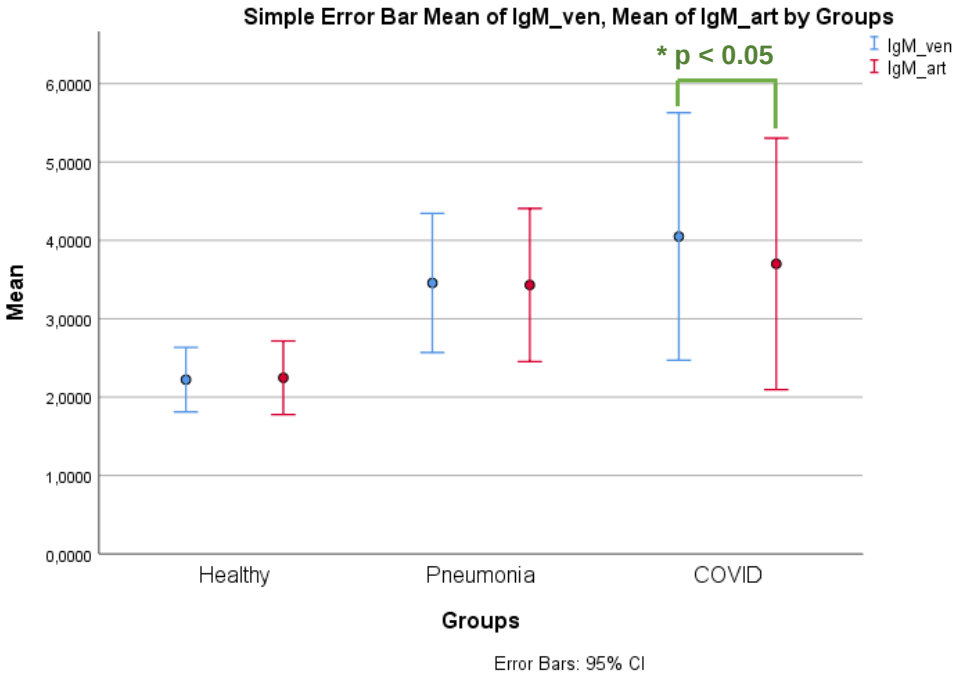

Figure 4. Comparing the venous and arterial IgM in three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of Error bars and presentation of the mean.

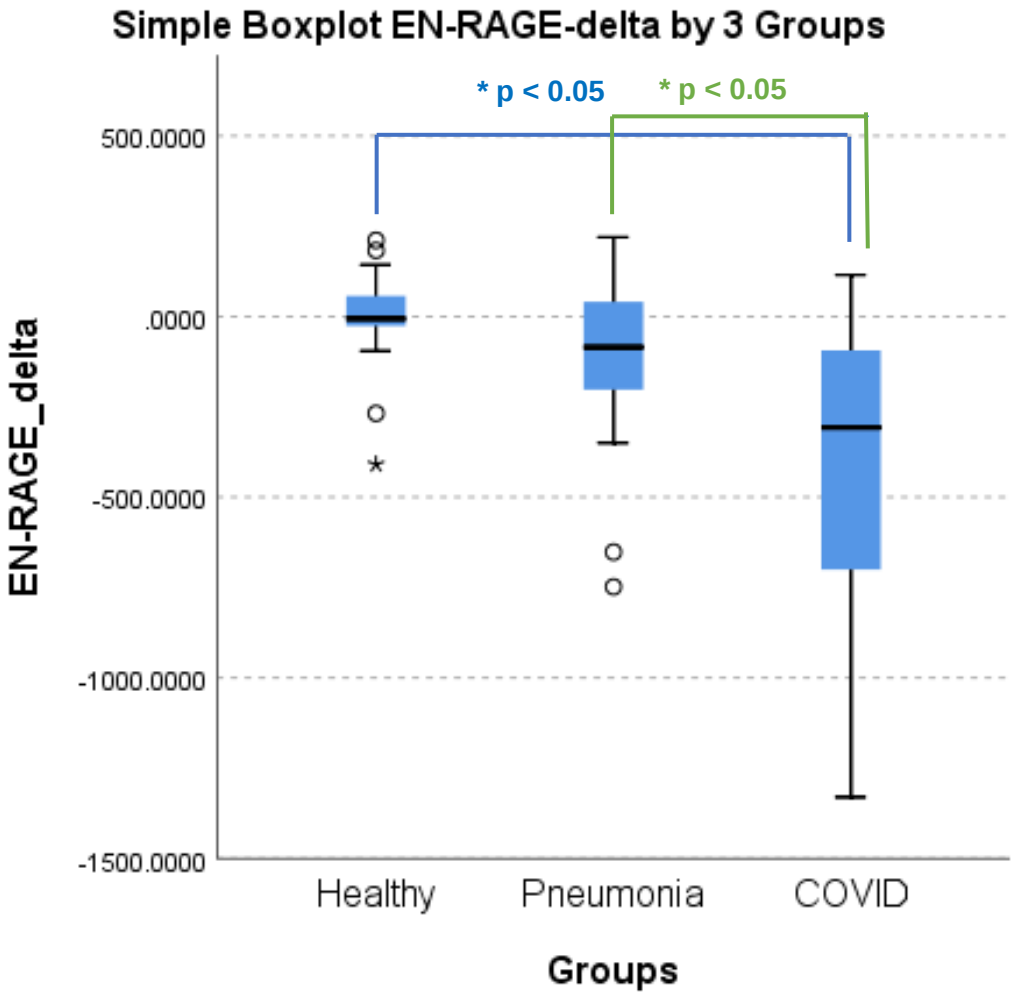

Figure 5. EN-RAGE-delta box plot comparing the three groups: control, pneumonia ARDS and COVID-19.

Performance of the comparison of the three groups by means of box plots and presentation of the medians, the quartiles and the outliers.

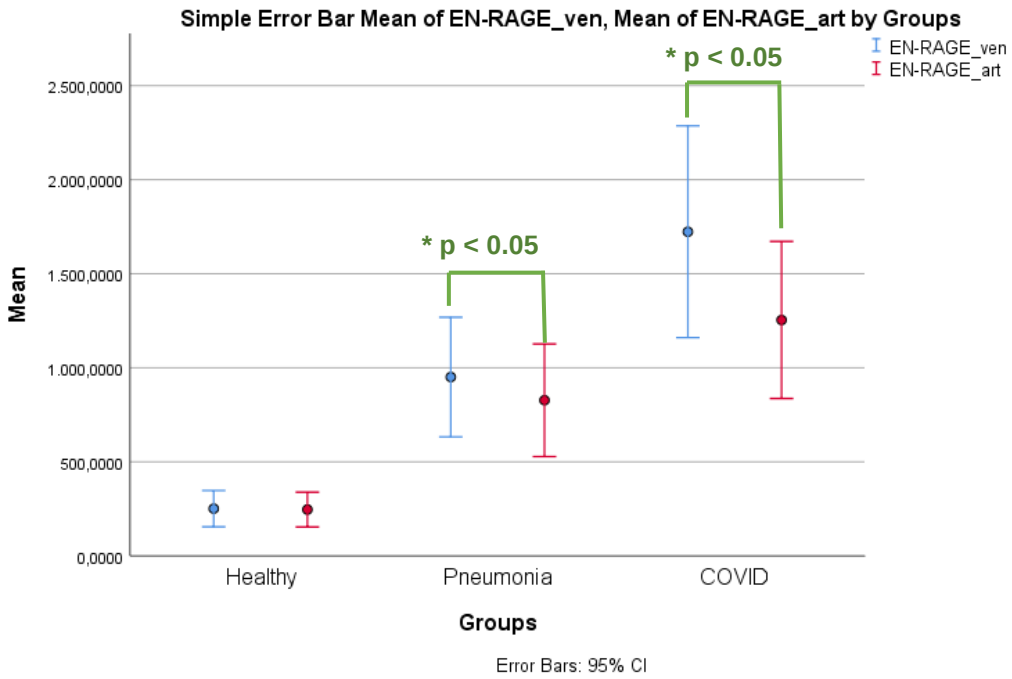

Figure 6. Comparing the venous and arterial EN-RAGE in three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of Error bars and presentation of the mean.

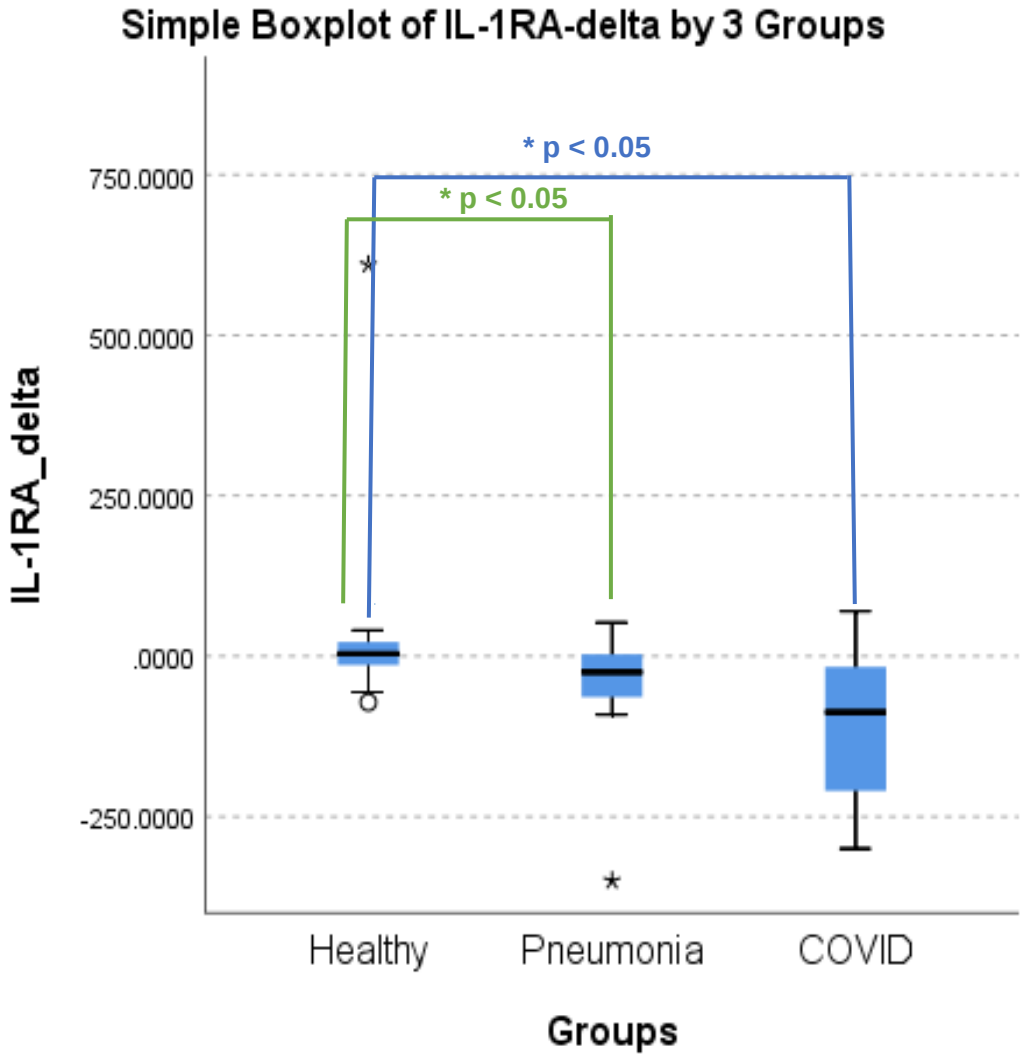

Figure 7. IL-1RA-delta box plot comparing the three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of box plots and presentation of the medians, the quartiles and the outliers.

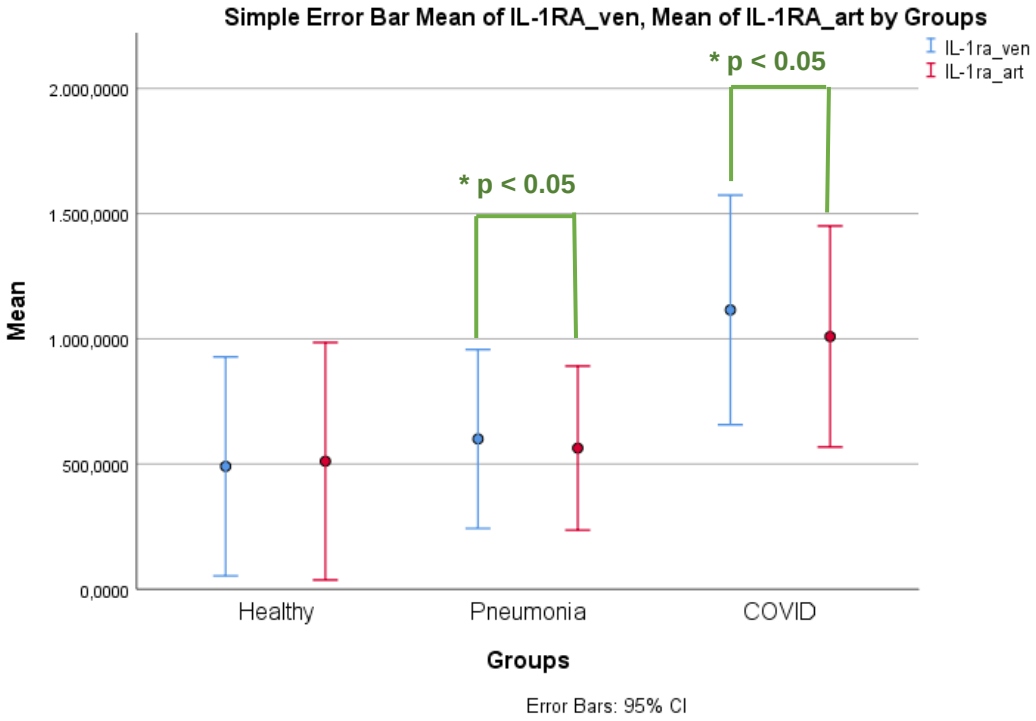

Figure 8. Comparing the venous and arterial IL-1RA in three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of Error bars and presentation of the mean.

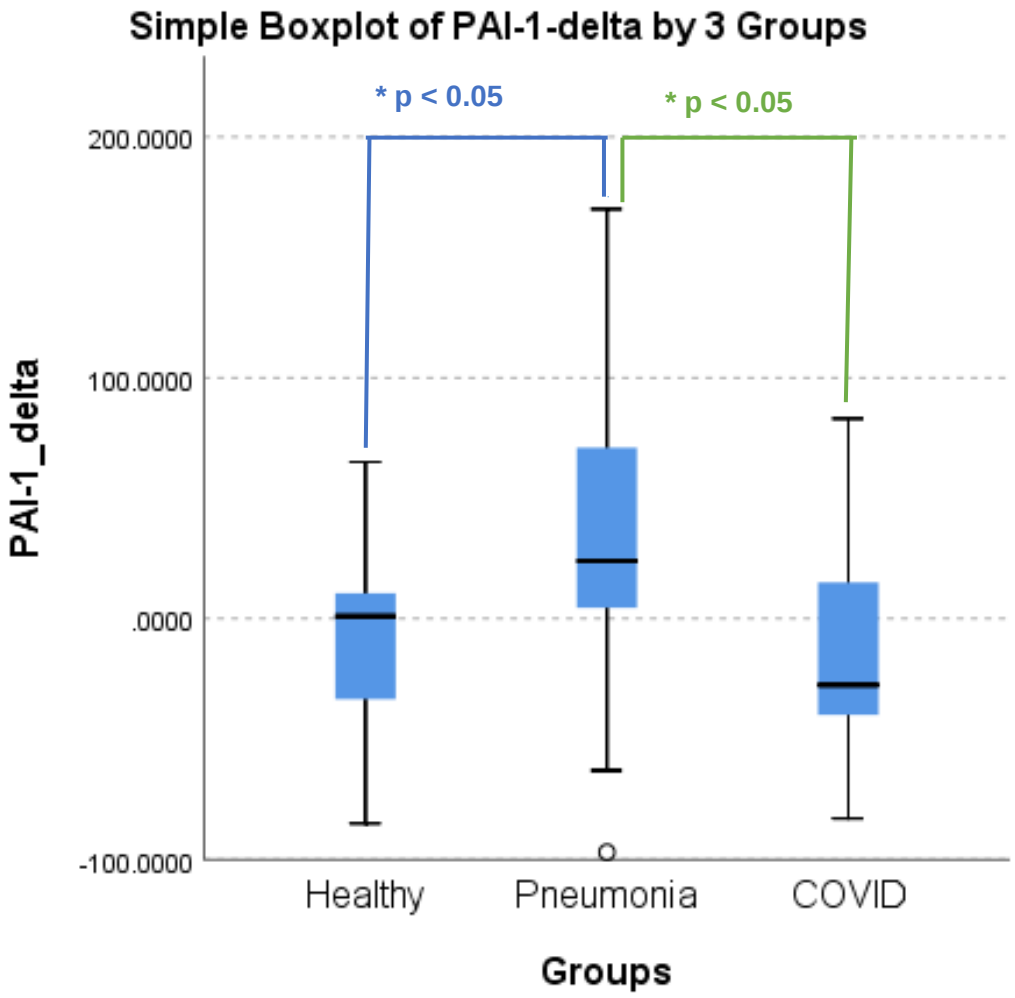

Figure 9. PAI-1-delta box plot comparing the three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of box plots and presentation of the medians, the quartiles and the outliers.

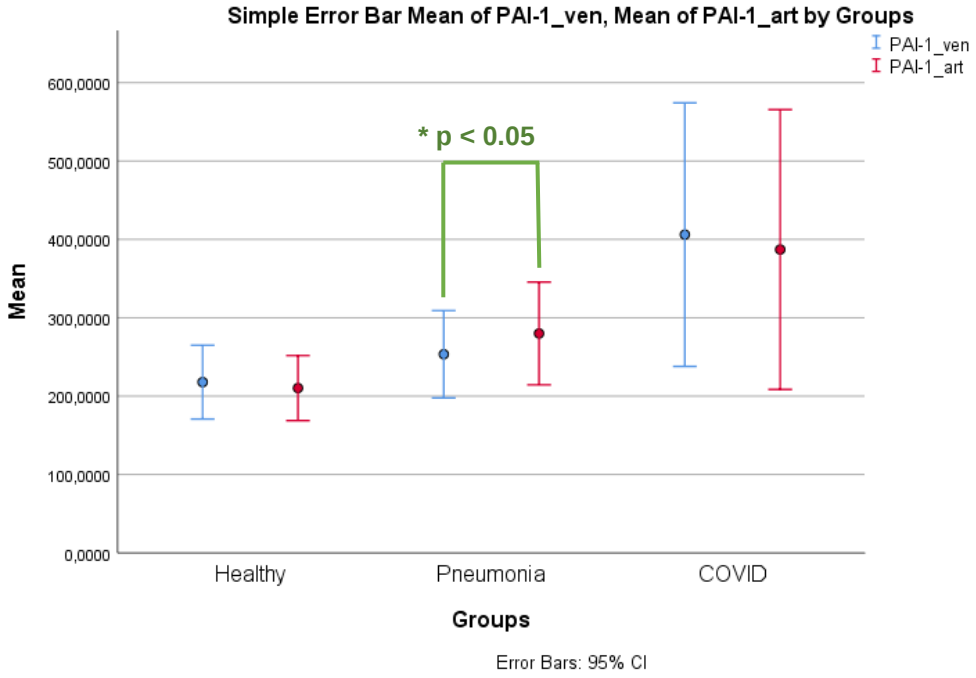

Figure 10. Comparing the venous and arterial PAI-1 in three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of Error bars and presentation of the mean.

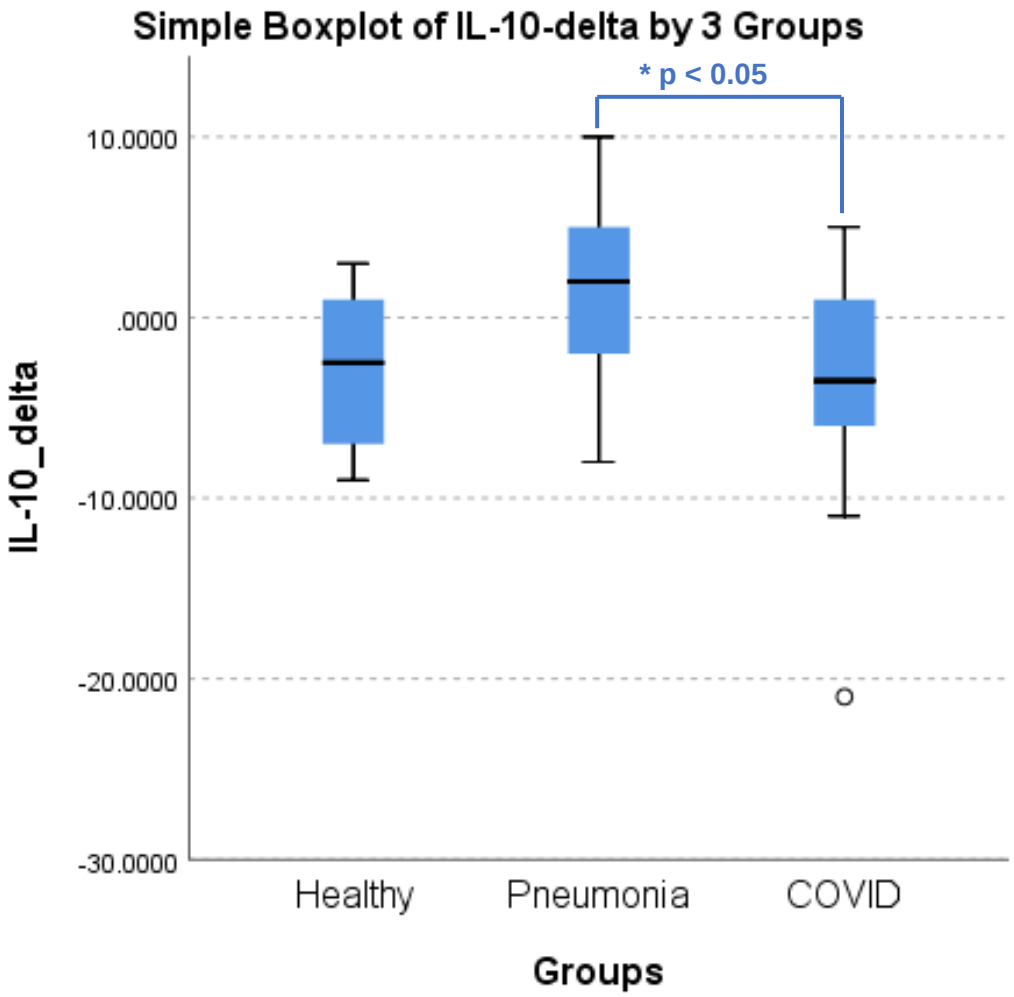

Figure 11. IL-10 box plot comparing the three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of box plots and presentation of the medians, the quartiles and the outliers.

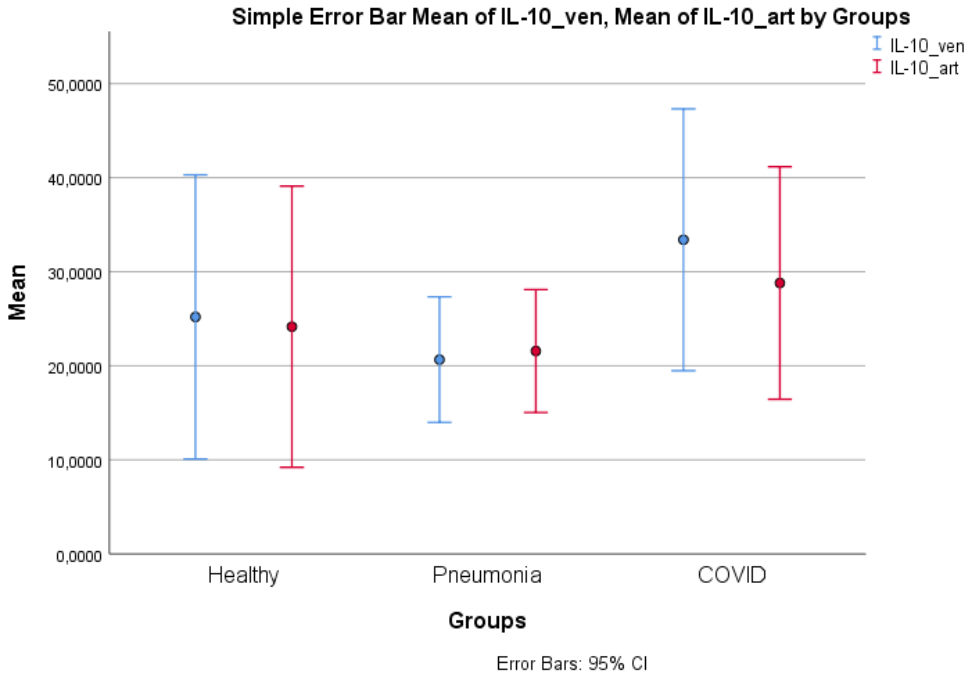

Figure 12. Comparing the venous and arterial IL-10 in three groups: control, pneumonia / ARDS and COVID-19.

Performance of the comparison of the three groups by means of Error bars and presentation of the mean.

4.4. Correlation of the cytokines with the SAPS and TISS

SAPS and TISS are scores that are used in intensive care medicine. The higher the respective values of the scores, the more complex the need for care and the more difficult the clinical course.

The SAP and TIS scores could only be determined for those patients who were in the intensive care unit at the time that blood was drawn. Thus, these scores could be determined in pneumonia / ARDS and COVID-19 groups.

In the case of a normal distribution, we applied the Pearson correlation coefficient, in the absence of a normal distribution the Spearman correlation coefficient was used. The significance value was $p<0.05$. Only the statistically relevant biomarkers are listed here.

The mean of the respective scores, divided into the different groups, are listed in Table 14 below for an overview.

			Mean	Maximum	Minimum
SAPS	2 Groups	Pneumonia	N=40,17	N=58,00	N=23,00
		COVID-19	N=38.70	N=56,00	N=22,00
TISS	2 Groups	Pneumonia	N=11,74	N=30,00	N=5,00
		COVID-19	N=14.30	N=23.00	N=5,00

Table 14. Means of the SAP and TIS scores.

The Mean of the two scores, divided into the two groups.

Table 15 shows the negative or positive correlation between the two scores and the relevant biomarkers. A negative correlation means that the higher the score, the lower the biomarker and vice versa.

In our study the biomarkers correlated with intensive scores, SAPS and TISS, in pneumonia / ARDS patients but not in COVID-19 group (table 15).

Cytokine	Scores	Spearman Correlation	Sig.
BDNF-delta	SAPS	-0.491	0.024
Albumin-delta	TISS	0.583	0.014
AAT-delta	TISS	-0.537	0.015
CRP-delta	TISS	-0.525	0.010
IL-6-delta	TISS	-0.565	0.012
TBG-delta	TISS	-0.460	0.031
CEA-delta	TISS	-0.481	0.023
Decortin-delta	TISS	-0.625	0.007
SPD	TISS	-0.481	0.027

Table 15. Correlation of intensive scores, SAP and TIS in pneumonia / ARDS group.

The individual relevant delta values of biomarkers are shown in their correlation with TISS and SAPS and the significance values.

4.5. Outcome of the COVID-19 group

In this analysis, the outcome was equated with the death of the patient. Only the COVID-19 group could be used for this evaluation, as the number of deceased patients in the pneumonia / ARDS group was not for a statistical analysis significant. In the COVID-19 group, 5 of 10 patients died in the course of the disease. Due to the small size of the COVID-19 group we assumed a normal distribution.

We looked for significant differences in biomarkers within the two subgroups, survivors and deceased. The significance value was $p < 0.05$.

Table 16 lists the biomarkers, which differed significantly in subgroups. All 3 listed biomarkers had a significantly higher means of delta values in the deceased patients than in the survivors.

Biomarkers	Significance values	Mean of Survivors	Mean of deceased	Difference
VCAM-1 in ng/mL	0.043	-84.60	94.50	7
PECAM-1 in ng/mL	0.026	-8.40	6.25	7
Decortin in ng/mL	0,019	-0.225	0.333	5

Table 16. Biomarkers that showed difference in the deceased patients and the survivors.

Significance values and the mean values of the subgroups deceased or survivors, as well as the differences between them. **L:** liter, **mL:** milliliter, **g:** gram, **mg:** milligram, **ug:** microgram, **ng:** nanogram, **pg:** picogram.

In the following figures 13, 14 and 15, the relevant biomarkers are shown as error bars.

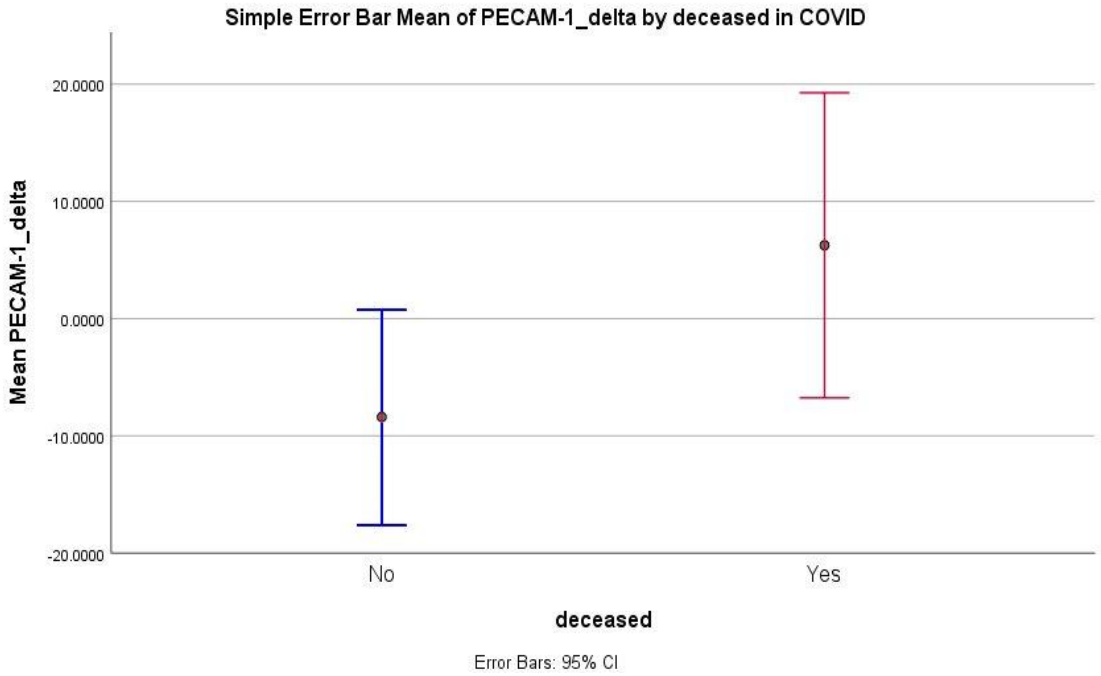

Figure 13. PECAM-1_delta by subgroups in COVID19.

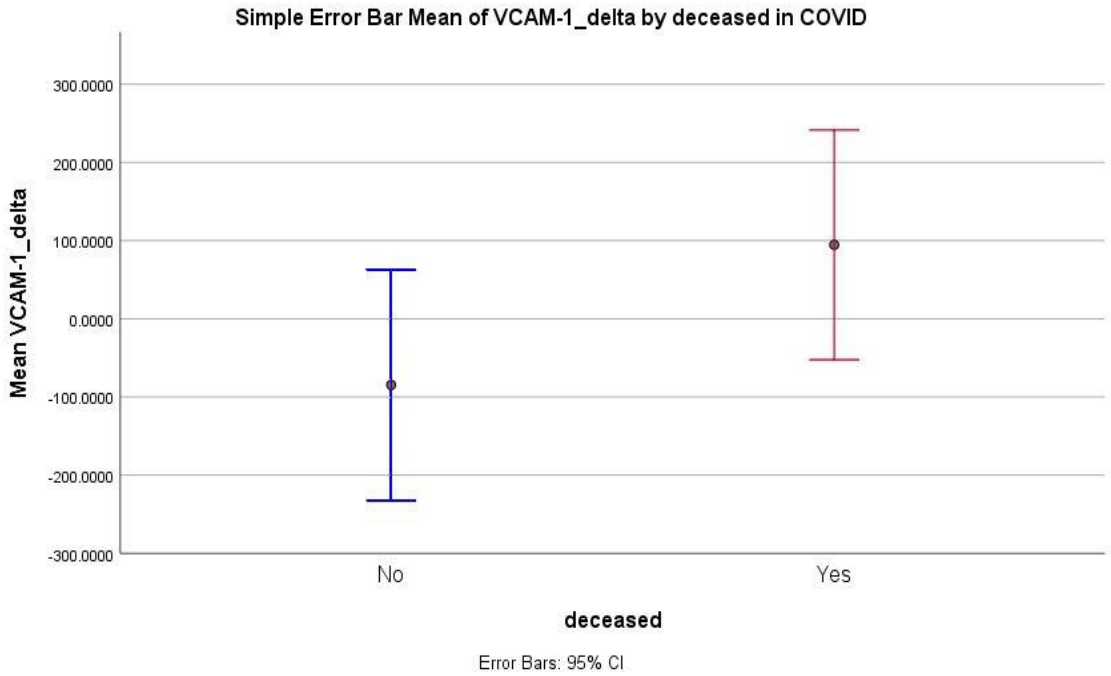

Figure 14. VCAM-1_delta by subgroups in COVID19.

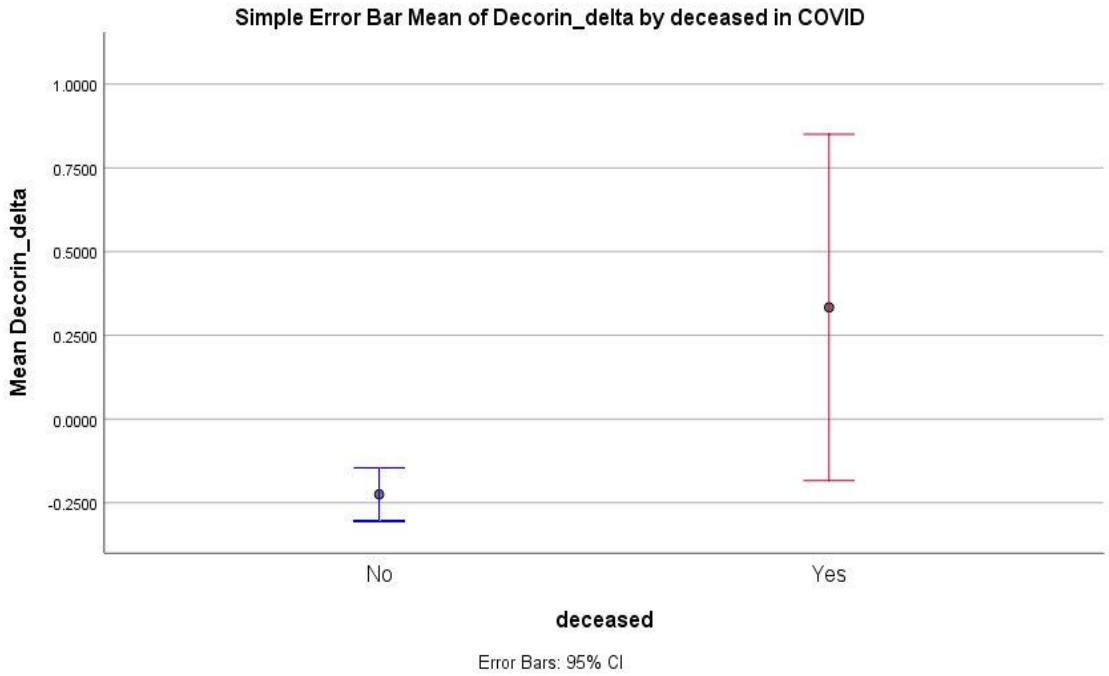

Figure 15. Decorin_delta by subgroups in COVID-19.

4.6. Biomarkers that showed no significance

Of 76 tested biomarkers, 56 showed no significance whatsoever for the questions and tests listed above. These are total protein, Adiponectin, A2-Macro, Lpa, B2M, C3, Eotaxin1, FRTN, Fibrinogen, GMCSF, Haptoglobin, ICAM1, IFNgamma, IL1alpha, IL2, IL3, IL4, IL5, IL7, IL8, IL12p40, IL12p70, IL17, IL18, MIP1alpha, MIP1beta, MMP3, MMP9, MCP1, Myoglobin, PARC, SAP, SCF, RANTES, TIMP1, TNFalpha, TNFbeta, TNFR2, VEGF, VDBP, vWF, AFP, ANG1, AXL, CA125, CA199, CA9, HCC4, FAS, HGF, hCG, IL18bp, MMP1, MMP7, MMP9 total und TRAILR3. . The concentrations of IL-2, IL-3, IL-5, GM-CSF and TNF-alpha were below the detection threshold.

5. Discussion

The aim of this work was to investigate how the lungs intervene in the serum concentration of certain biomarkers in different disease settings and to investigate if lung secretes inflammatory cytokines into the systemic circulatory system in two different groups of patients, pneumonia / ARDS and COVID-19. We compared the transpulmonary gradients of biomarkers among three different groups: lung-healthy individuals, pneumonia / ARDS and COVID-19 groups.

Some biomarkers showed statistically significant differences in delta values among three groups. The biomarkers with statistically relevant differences among three groups of patients, were tested further for relevant differences between venous and arterial samples within each group. The concentrations of these biomarkers were higher, both venous and arterial, in COVID-19 patients, indicating a higher inflammatory state in comparison to pneumonia / ARDS group.

The comparison between the surviving and deceased COVID-19 patients revealed significant differences for the delta values of 3 biomarkers (VCAM-1, PECAM-1 and Decortin), two of these biomarkers were cell adhesion molecules. We ran a paired t-test on the venous and arterial samples of these biomarkers, as well. Based on higher arterial concentrations of these biomarkers in deceased patients, we assume that in deceased patients the activation of the pulmonary endothelial cells through SARS-CoV-2 leads to shedding of these two CAMs from endothelium.

The COVID-19 was the subject of many studies due to their great global importance in 2020 and 2021. There are many publications on COVID-19 and biomarkers [1,6,17,19,39,42]. There has been a growing interest in the application of novel biomarkers that appear to be promising for the accurate diagnosis and risk stratification of pneumonia and ARDS.

Our study examined a total of 76 biomarkers to identify further significant correlation of biomarkers in different disease settings.

It should be noted that many aspects of our study have not yet been investigated, especially the transpulmonary gradient of the biomarkers.

5.1. Comparison of the groups

Four biomarkers differed significantly between the COVID-19 and the control groups. The delta values of all these biomarkers were lower in COVID-19 group. These included NSE, IgM, EN-RAGE, and IL-1RA.

Two biomarkers were identified with significant differences in the delta values between pneumonia / ARDS and control groups. Of these, the delta value of PAI-1 was higher and IL-1RA was lower in the pneumonia / ARDS group.

The comparison between the COVID-19 and the pneumonia / ARDS groups resulted in 3 relevant biomarkers between the two groups. All of them had a higher delta value in pneumonia / ARDS group. These included PAI-1, IL-10 and EN-RAGE.

We could not find studies comparing the transpulmonary gradient of biomarkers, thus we are not able to compare our study with others. Furthermore, it is not obvious, whether the negative gradient of the biomarkers through the pulmonary passage caused by pulmonary uptake or by systemic secretion of the biomarkers and vice versa.

However, there are studies about transpulmonary concentration gradient of different medication, especially anesthetics [76]. Drugs with a substantial pulmonary uptake are basic amines with a pKa of > 8. In the group of basic amines, the pKa and lipophilicity are essential factors of lung uptake [88]. Molecular mass, hydrophobicity, protein binding, oxygenation, ventilation, concomitant drugs, cardio-pulmonary bypass, lung pathology, ageing, perfusion, and anesthesia are also known factors to control the pulmonary uptake [89]. None of the drugs with high pulmonary extraction experience substantial metabolism within the lung. As the systemic levels diminish, drug is released from the lung.

5.1.1. IL-1RA

Interleukin-1 receptor antagonist (IL-1RA) is secreted naturally to impede the effect of Interleukin-1 (IL-1) and inhibit the proinflammatory effect of IL-1β, by competitively binding to IL-1 receptor I (IL-1RI). It secretes simultaneously with IL-1, which is commonly increased at the stages of inflammation such as lung injury [90].

Genomic deletion of IL-1RA lets IL-1β unconstrained and therefore causes fetal systemic inflammation [91]. When lung damage occurs, IL-1 releases prompting inflammation and IL-1RA releases to confront this process. Treatment with recombinant IL-1RA diminishes pulmonary fibrosis and pneumonia in animal models [92].

Liu et al. showed that 38 cytokines in the immature plasma cells (plasmablasts) of COVID-19 patients were significantly raised, and 15 cytokines involving IL-1RA were linked with severity of disease [93].

Our study showed a significant decrease in delta value of IL-1RA in COVID-19 and pneumonia / ARDS groups compared to the control group, but no relevant difference between the two groups of pneumonia / ARDS and COVID-19. However, The means of both arterial and venous

IL-1RA concentrations were the highest in COVID-19 group and the lowest in the control group, which could indicate a higher state of pulmonary inflammation in comparison to other types of ARDS. The paired t-test on arterial and venous concentrations of IL-1RA was in pneumonia / ARDS and COVID-19 groups statistically significant with a lower concentration in arterial samples in both groups, eventually as an indicator for binding to IL-1 receptor at the inflammation site.

From the therapeutic perspective, Anakinra, a recombinant IL-1 receptor antagonist, might help to counteract the severe acute respiratory syndrome. Huet et al. reported in a cohort study that Anakinra significantly decreased both need for invasive mechanical ventilation in the ICU and mortality among patients with severe COVID-19, without significant side-effects [94].

5.1.2. IL-10

Interleukin-10 (IL-10) is an anti-inflammatory cytokine, and it was also confirmed that IL-10 protects lung from damaged induced by lipopolysaccharide (LPS) [95].

In our study there was a significant difference between the delta values of IL-10 in COVID-19 and Pneumonia / ARDS groups, with a lower mean in COVID-19 group. Although the means of both venous and arterial IL-10 concentration were higher in COVID-19 group than in pneumonia / ARDS group, probably it could act as an indicator for the body response to the elevated levels of proinflammatory mediators in COVID-19 patients. Within the COVID-19 group, we found a lower mean of arterial concentration than venous. Contrarily, within the pneumonia / ARDS group, the venous concentration was lower. The underlying cause is unknown.

Several studies [96, 97–99] have found IL-6, IL-10, IL1RA, and IL-13 as endogenous regulators in the acute inflammatory response in lung. Lentsch et al. [100] reported that the exogenously administration of IL-4, IL-10, or IL-13 significantly reduced the lung injury induced by IgG immune complexes.

5.1.3. EN-RAGE

In the lung, Receptor for Advanced Glycation End products (RAGE) expression is predominantly located on the basal surface of alveolar type I cells [101, 102]. However, RAGE is generated by other cell types in different systems, such as vascular endothelium and neural tissues. Hence, it is not specific for alveolar type I cell injury. Yet, it is most abundant in the lung and its plasma concentrations largely indicate epithelial injury. Therefore, it seems to be appropriate as a marker for the extent of ventilator-associated lung injury [103, 104].

Extracellular newly identified RAGE-binding protein (EN-RAGE) purified from bovine lung extract has been reported as a ligand for RAGE.

In our trial, the differences of delta values of EN-RAGE were significant between COVID-19 and control groups as well as between COVID-19 and pneumonia / ARDS groups. The COVID-19 group had the lowest mean of delta value, but the means of both venous and arterial EN-RAGE concentrations were the highest in COVID-19 group followed by pneumonia / ARDS group. Applying paired t-test, we found out that the arterial mean of EN-RAGE was lower in both COVID-19 and pneumonia / ARDS groups.

An analysis of direct and indirect ARDS in two clinical cohorts reported that patients with direct lung injury had higher plasma levels of lung epithelial damage biomarkers (such as RAGE and surfactant protein D), while patients with indirect lung injury had more elevated plasma levels of endothelial injury biomarkers such as angiopoietin 2 [105].

In another study from Calfee et al, RAGE was measured and it showed that lung-protective ventilation reduced alveolar epithelial cell injury in comparison with traditional tidal volumes [106]. We did not consider the ventilation parameters of patients in our analysis; thus, we are not able to compare our results with this study. Furthermore, the reason of a lower arterial concentration of EN-RAGE comparing to venous concentration is not clear for us.

5.1.4. NSE

Neuron-specific enolase (NSE) is a glycolytic enzyme that is expressed in the cytoplasm of neurons and neuroendocrine cells. It is present predominantly in the amine precursor uptake and decarboxylation (APUD) system, such as in the pituitary, thyroid, pancreas, intestine, and lung [107]. NSE is employed as a tumor marker in the diagnosis, prognosis, and follow-up of small-cell lung carcinoma [108]. Clinical evidence emphasizes the role of NSE in the diagnosis, therapy, and monitoring of both acute and chronic lung damages [109, 110], solitary pulmonary nodules [111], and infectious lung illnesses such as tuberculosis [112]. Furthermore, NSE is known to stimulate the synthesis of proinflammatory mediators [113].

Cione et al. assessed serum NSE levels in COVID-19 patients with and without dyspnea. In this study, they investigated both SARS-CoV2-infected and non-infected patients older than 18 years old who were admitted to hospitals in Catanzaro, Italy from March 30 to July 30, 2020. They found significantly higher NSE values in COVID-19 patients than in control group. Remarkably, within the COVID-19 group, they also found an additional significant increase in symptomatic patients with dyspnea [114].

In our trial, the mean of delta value in COVID-19 group was in comparison to control group lower. Applying paired t-test higher means in both venous and arterial samples were seen, most likely in response to inflammation.

5.1.5. IgM

The delta value of IgM in COVID-19 group was statistically lower than the control group. However, the concentrations of both venous and arterial IgM were higher in COVID-19 group in comparison to the other groups. In paired t-test the only significant difference was seen in the COVID-19 group. We could not find similar studies comparing the transpulmonary gradient of IgM.

5.1.6. PAI-1

In the lung, Plasminogen activator inhibitor-1 (PAI-1) is produced mainly by the alveolar macrophages, alveolar type II cells and fibroblasts [115-117].

As appears in acute lung injury, activation of coagulation in sepsis is related with depressed systemic fibrinolysis [118]. In opposition, systemic abnormalities of fibrin turnover can take place in connection with acute lung injury. For example, the plasma concentration of PAI-1 is susceptible to be more elevated in patients with ARDS than in seriously sick control patients [119]. This observation indicates that local abnormalities of fibrinolysis in ARDS may be manifested in the systemic circulation.

In COVID-19 disease, the virus attacks the host cells whereas ACE2 is internalized and incapable to induce the breakdown of angiotensin II. The excess of angiotensin II causes an increase of PAI-1 and reduced fibrinolysis [120–122], generating a hypercoagulable state, whereas the excess of angiotensin II binds to its receptor angiotensin II receptor 1a, prompting lung damage and.

In our study, the delta values differed significantly between control and pneumonia / ARDS groups as well as in COVID-19 and pneumonia / ARDS groups. The delta value was the lowest in COVID-19 group. However, the means of both venous and arterial samples were higher in COVID-19 group comparing to pneumonia / ARDS group. In paired t-test, the difference was only in the pneumonia / ARDS group statistically significant with a higher arterial concentration of this biomarker, most likely indicating its release from alveolar cells.

In one study on sera of patients with COVID-19, angiogenic cytokines in four groups were evaluated: healthy control, COVID-19 hospital admitted, COVID-19 survived ICU admitted, and COVID-19 non-survived patients. They reported that angiopoietin 2 (Ang-2) and plasminogen activator inhibitor 1 (PAI1) were significantly more elevated in non-survivors in

comparison to survived ICU patients. In the survived ICU patients, levels of Ang-2, endoglin (ENG), fibroblast growth factor 1 (FGF-1), Fms-related tyrosine kinase 3 ligand (FLT-3L), and PAI-1 were significantly greater than the ward admitted COVID-19 patients. Moreover, sera levels of VEGF-A, PDGF-AA, PDGF-AB/BB, PAI-1 were significantly more elevated in the ward admitted COVID-19 patients in comparison to the healthy controls. PAI-1, on the other hand, was identified exceptionally elevated upon worsening of COVID-19 patients' clinical status [123].

5.2. Correlation of the intensive care medicine scores (SAPS and TISS) with the delta values of biomarkers in pneumonia / ARDS and COVID-19 groups

Another question in our study was the correlation between the two intensive care medicine scores SAPS and TISS and the delta values of biomarkers in pneumonia / ARDS and COVID-19 groups. We found a correlation between the two scores and pneumonia / ARDS for some metabolites. There are currently no studies on this subject. Yet, we do not know if these results would have a therapeutic relevance, further studies should be done.

5.3. Outcome of the COVID-19 group

There were three biomarkers with significant differences of delta values between survivors and deceased patients. As demonstrated in Table 17, the means of delta values of all these three biomarkers (VCAM-1, PECAM-1 and Decortin) were positive in deceased patients, whereas they were negative in survivors, which shows a higher arterial concentration of these biomarkers in deceased patients. Conversely, the venous concentrations of these biomarkers were higher than arterial blood in the survivors.

We could not find similar studies, which compare the concentration of the biomarkers through the lung passage and examine the correlation of the delta values of the biomarkers with the patient’s outcome. Therefore, a direct comparison between our results with other studies will not be possible. However, there are studies which have shown that there are some correlations between biomarkers concentration, especially cell adhesion molecules, and the outcome of COVID-19 patients.

VCAM-1 and PECAM-1 are cell adhesion molecules. Leukocytes can circulate as non-adherent cells in the blood stream, and upon activation of the CAM, they can adhere to the vascular endothelial cells and migrate into the target tissues via the intercellular junctions between these cells [124, 125].

VCAM-1 is the main ligand for VLA-4 [126-129], which is expressed mainly on lymphocyte, monocytes, and eosinophils, but is not found on neutrophils. VLA-4 interacts with its ligands VCAM-1 and fibronectin (FN) CS1 during chronic inflammatory diseases.

Platelet endothelial cell adhesion molecule 1 (PECAM-1 or CD31) is a highly glycosylated immunoglobulin-like membrane receptor expressed by leukocytes, platelets, and especially endothelial cells. In addition, PECAM-1 is a marker of endothelium [130]. Just like ICAM-1 and VCAM-1, the extracellular domain of PECAM-1 functions to mediate cell-cell interactions and give rise to a tight barrier of the endothelium [131, 132]. Elevation of soluble PECAM-1 (sPECAM-1) level has also been shown in the serum of patients with myocardial infarction, acute ischemic stroke, and multiple sclerosis, conditions that involve tissue damage and endothelial cell apoptosis [133, 134].

We did not observe any significant correlation between the delta value of ICAM-1 and the outcome of SARS-CoV-2-disease. Bloemen et al. suggested that only ICAM-1 was upregulated by inflammatory cytokines during the inflammation of air epithelium [135]. However, VCAM-1 expression was also observed on activated human bronchial epithelial cells, suggesting a selective recruitment of VLA-4-positive cells (e.g., eosinophils) into the inflamed airway epithelium [136].

CAM are also directly involved in viral (e.g., rhinovirus and HIV) infection processes. For instance, ICAM-1 is a major receptor in rhinovirus infection of the airways, causing airway inflammation that can progress to asthma [137]. After the initial viral infection, upregulation of ICAM-1 expression on the pulmonary epithelial cells is stimulated by cytokines released by the epithelial cells; this ICAM-1 upregulation could thus facilitate further rhinovirus infection [138]. The similar function could have been possible for other CAM in other viral diseases such as COVID-19. Therefore, VLA-1, for instance, may be an important target for inhibition of certain pulmonary viral infections like COVID-19.

Tong et al demonstrated that the increased expression of endothelial cell adhesion molecules is correlated with COVID-19 severity and may contribute to coagulation dysfunction [139]. The authors examined the expression of 3 endothelial cell adhesion molecules by enzyme-linked immunosorbent assays (ELISA), including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion protein-1 (VAP-1). Serum levels of fractalkine, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and vascular adhesion protein-1 (VAP-1) were elevated in patients with mild disease, dramatically elevated in severe cases, and decreased in the convalescence phase. They concluded the increased expression of endothelial cell adhesion molecules is related to COVID-19 severity and may contribute to coagulation dysfunction [139].

Li et al showed that the serum levels of sPECAM-1 were not only significantly higher in COVID-19 patients than in healthy controls but also significantly higher than in asymptomatic carriers. In addition, the serum levels of sPECAM-1 were positively correlated with disease severity, and sPECAM-1 levels in patients in critical disease were significantly higher than in patients in moderate or severe condition. These results demonstrated that SARS-CoV-2 infection alone is not enough to stimulate endothelial cell activation, which led to sPECAM-1 shedding from endothelium [140].

None of the studies above compared the concentrations of the CAM in arterial and venous samples, thus again a direct comparison between these studies and ours is not possible. In our project, the difference of delta values of VCAM-1 and PECAM-1 were statically significant and contribute to the outcome of the patients. The mean of the delta values of the biomarkers were positive in deceased patients, which shows a higher concentration of these biomarkers in arterial samples. One hypothesis for the higher arterial concentrations of these two CAMs could be explained through the activation of the pulmonary endothelial cells through SARS-CoV-2, which lead to shedding the CAMs from endothelium or upregulation of the CAM on the leukocytes through lung passage. Furthermore, the delta-Concentration of Decortin was attributed to the outcome of our Corona-patients, which has not been studied before.

5.4. Limitations

The number of COVID-19, pneumonia / ARDS patients and patients with healthy lungs included was relatively low in absolute terms. The arterial samples were not taken directly after the lung passage via a pulmonary arterial catheter, thus it is not conclusive if the transpulmonary gradient of biomarkers primarily caused by uptake or secretion in the lung or it is a systemic issue. Furthermore, the time period between diagnosis of the diseases and the inclusion of the patients in our study was variable.

5.5. Conclusion

Cytokines and biomarkers can be used to identify mechanisms of the disease and the course of COVID-19 and pneumonia / ARDS patients. Some of the tested biomarkers such as CAMs have the potential to play an essential role in the prognosis of the disease in the future.

Furthermore, the lung likely influences the concentration of some biomarkers in blood similar as it does on serum concentration of certain medication specially anesthetic drugs.

6. Index

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6.2. List of tables

6.3. List of figures

7. Appendix

Cytokine	Function
Adiponectin	Increase in insulin sensitivity [70].
AAT	Acute Phase Protein [70]
A2Macro	Ion transport, inhibition of proteinases [141]
AFP	Discovery and assessment of the course of hepatocellular carcinoma [142]
ANG-1	Angiopoetin 1 produced by vascular smooth-muscle cells and is responsible for stabilization and maturing of the vessels [143]
Lp(a)	Apolipoproteins make lipids transportable [70]
AXL	Tyrosine Kinase Receptor [144]
B2M	Expression by the lymphatic system in inflammation, immune diseases and viral infections [145]
BDNF	Modulates cognition, neuroplasticity, angiogenesis and neural connectivity [146]
CA-125	Related to ovarian cancer, carcinoma of the gastrointestinal tract, lungs or breast, as well as to inflammatory processes of the adnexa [147]
CA-19-9	Related to various carcinomas of the gastrointestinal tract, pancreatic and hepatobiliary carcinomas and adenocarcinomas of other origins [148]
CA-9	pH regulation, expressed by a variety of solid tumors [149]
CEA	Causes the activation and production of pro- and anti-inflammatory cytokines, colon cancer [150,151]
HCC-4	Chemokine for monocytes and dendritic cells [150,152]

C3	Acute phase protein [70]
CRP	Acute phase protein [70]
Decorin	Control of the fibrillogenesis of collagens [150]
EN-RAGE	Receptor for advanced glycation end products (RAGE) expression in the lung is primarily located on the basal surface of alveolar type I cells [101-104]
Eotaxin	Strong chemokine for eosinophils [153]
Faktor VII	Factor VII belongs to the external coagulation pathway [70]
FAS	Part of the induction of apoptosis [150]
FRTN	Ferritin is known as iron storage protein [70]
Fibrinogen	Acute phase proteins, adhesive protein from fibronectin, vWF, thrombin, heparin and calcium ions [70]
GM-CSF	Activation of granulocytes, monocytes and antigen presenting cells [154]
Haptoglobin	Is the transport protein for hemoglobin in the blood and thus a hemolysis parameter [70]
HGF	Induces the mitogenesis of various epithelial cells, stimulates cellular motility and has angiogenic and anti-apoptotic effects [150]
hCG	Formed by the placenta during pregnancy and is more common in trophoblastic tumors [150]
IgA	Synthesis in the B cells, mucous membrane barrier, increased IgA values are common in skin, intestinal, respiratory and kidney infections [150,155]
IgM	Immunoglobulin of the primary response to infections, for agglutination of the pathogens and activation of the classic pathway of the complement system [150]

ICAM-1	Activation of lymphocytes, adhesion molecule [150]
IFN gamma	Activation of macrophages, inhibit all stages of virus replication [70]
IL-1 alpha	Proinflammatory spectrum of activity [150]
IL-1 beta	Proinflammatory spectrum of activity [150]
IL-1RA	Blocks IL-1 receptors and thus acts as a natural anti-inflammatory [108]
IL-2	Activation of T cells [70]
IL-3	Immunomodulatory cytokine, important stimulation of T cell differentiation [70]
IL-4	Anti-inflammatory and immunomodulatory cytokine and involved in the formation of immunoglobulins [70]
IL-5	Involved in the formation of immunoglobulins [70]
IL-6	Proinflammatory, anti-inflammatory and immunomodulatory cytokine [70]
IL-7	Immunomodulatory cytokine [70]
IL-8	Is a chemokine and is therefore essential to the inflammatory reaction [70]
IL-10	Anti-inflammatory cytokine [70]
IL-12 p40	Proinflammatory cytokine [150]
IL-12 p70	Proinflammatory cytokine [150]
IL-17	Proinflammatory cytokine[150]
IL-18	Mediates the T-helper polarized immune response and promotes inflammation by increasing the production of TNF alpha and IFN gamma [156]
IL-18bp	Antagonist of IL-18 [157]

MIP-alpha	Chemokine [150]
MIP-beta	Chemokine [150]
MMP	Matrix metalloproteinases are also known as collagenases and are responsible for breaking down collagen [70]
MCP-1	Chemokine for lymphocytes and monocytes[150]
Myoglobin	Myoglobin is structurally homologous to a hemoglobin subunit and is the oxygen storage protein of a muscle cell [70]
NSE	Found in neurons of the brain and in endocrine tissue, especially in the APUD cells in the intestine, the lungs and endocrine organs and are particularly in demand for neuroendocrine tumors [107-113]
PAI-1	Negative feedback on fibrinolysis with inhibition of coagulation [133-137]
PECAM-1	Leads to the adhesion of leukocytes to the endothelium [130-133]
PARC	Chemokine für lymphocytes [158]
SP-D	Reduces the surface tension of the lungs, involved in immune and inflammatory regulation of the lungs [159]

Table 17. List of all tested biomarkers and their function

			Mean	Standard Deviation	Median	Range
Alb_delta	Groups	Control	-,0909	2,0681	-1,0000	8,0000
		Pneumonia	2,4118	8,1705	1,0000	35,0000
		COVID-19	2,1111	7,7208	2,0000	22,0000
GEW_delta	Groups	Control	,1905	3,6826	1,0000	13,0000
		Pneumonia	3,0500	14,4858	-1,0000	66,0000
		COVID-19	3,3333	15,2807	-1,0000	44,0000
Adiponectin_delta	Groups	Control	-,0840	,8214	,1000	3,2000
		Pneumonia	,0957	1,2608	-,1000	5,0000
		COVID-19	-,1444	,4362	-,1000	1,5000
AAT_delta	Groups	Control	-,0257	,4075	-,1000	1,6000
		Pneumonia	,0050	,8763	-,1500	3,6000
		COVID-19	-,4222	,7710	-,5000	2,3000
A2Macro_delta	Groups	Control	,0455	,6139	,0500	2,4000
		Pneumonia	-,2227	,7597	-,1500	3,1000
		COVID-19	,0789	,2983	,2000	1,0000
Lp(a)_delta	Groups	Control	-6,0591	32,4131	,8500	146,0000
		Pneumonia	-29,8182	110,0972	-2,5000	582,0000
		COVID-19	4,0000	23,9344	-4,0000	74,0000
B2M_delta	Groups	Control	-,0450	,4582	-,1000	2,0000
		Pneumonia	-,3100	2,1094	-,2000	10,0000
		COVID-19	-,3000	,4989	-,2500	1,8000

BDNF_delta	Groups	Control	-,2684	2,2774	-,5000	10,5000
		Pneumonia	,2589	1,5914	,2000	6,8000
		COVID-19	,3650	,9493	,4000	2,7000
CRP_delta	Groups	Control	-2,0108	9,4620	-,1350	46,0000
		Pneumonia	-7,6957	34,0634	-4,0000	165,0000
		COVID-19	-24,6000	70,9322	-22,5000	267,0000
C3_delta	Groups	Control	-,0319	,2556	-,0200	1,1300
		Pneumonia	-,0668	,2218	-,1000	,8000
		COVID-19	-,0378	,1861	-,1000	,4500
EN-RAGE_delta	Groups	Control	-4,7692	125,2234	-5,5000	620,0000
		Pneumonia	-123,2609	238,6603	-85,0000	968,0000
		COVID-19	-469,2000	496,6344	-307,0000	1445,0000
Eotaxin-1_delta	Groups	Control	-15,4211	58,2297	-13,0000	217,0000
		Pneumonia	4,6667	71,7910	-14,0000	258,0000
		COVID-19	-15,8571	79,2095	-21,0000	204,0000
Factor VII_delta	Groups	Control	,1200	25,7881	1,0000	92,0000
		Pneumonia	-4,1739	37,9792	-2,0000	176,0000
		COVID-19	-16,3000	21,4012	-15,5000	66,0000
FRTN_delta	Groups	Control	24,8400	169,2229	21,0000	930,0000
		Pneumonia	81,1739	323,1415	48,0000	1660,0000

		COVID-19	-246,5556	631,3195	-220,0000	2040,0000
Fibrinogen_delta	Groups	Control	-,0032	,0070	-,0035	,0240
		Pneumonia	-,0091	,0421	,0010	,1860
		COVID-19	-,0046	,0098	-,0040	,0270
Haptoglobin_delta	Groups	Control	,0598	,5508	,0600	2,2000
		Pneumonia	-,2229	,7943	,1000	3,0000
		COVID-19	-,2875	2,1424	-,2000	7,0000
IgA_delta	Groups	Control	-,0542	,5388	-,0500	2,6000
		Pneumonia	-,2025	,4797	-,2000	1,6000
		COVID-19	-,5444	,6064	-,3000	1,7000
IgM_delta	Groups	Control	,0295	,3385	,1000	1,2000
		Pneumonia	-,0273	,4278	-,1000	2,0000
		COVID-19	-,3889	,4400	-,3000	1,6000
ICAM-1_delta	Groups	Control	-,3600	33,1145	4,0000	159,0000
		Pneumonia	-12,1739	75,7692	-4,0000	375,0000
		COVID-19	-105,7778	167,2586	-17,0000	449,0000
IL-1 beta_delta	Groups	Control	,2056	3,5541	,8000	11,7000
		Pneumonia	,2167	3,0438	,0000	10,0000
		COVID-19	-1,7222	2,1592	-1,6000	6,1000
IL-1RA_delta	Groups	Control	21,2400	126,3973	4,0000	682,0000
		Pneumonia	-39,9524	81,8227	-25,0000	402,0000

		COVID-19	-106,3000	122,5698	-87,5000	370,0000
IL-5_delta	Groups	Control	-8,5000	.	-8,5000	,0000
		Pneumonia	-3,0000	5,6569	-3,0000	8,0000
		COVID-19	.	.	.	.
IL-6_delta	Groups	Control	-3,1545	5,4257	-5,0000	15,4500
		Pneumonia	-2,0263	7,8304	-1,0000	39,0000
		COVID-19	-20,2000	38,8009	-3,0000	127,0000
IL-8_delta	Groups	Control	1,3792	13,9171	-2,0000	74,0000
		Pneumonia	-,6818	14,2641	1,0000	62,0000
		COVID-19	-7,2222	23,7370	4,0000	73,0000
IL-10_delta	Groups	Control	-2,7000	4,4981	-2,5000	12,0000
		Pneumonia	1,1667	5,2496	2,0000	18,0000
		COVID-19	-4,6000	7,2449	-3,5000	26,0000
IL-12p40_delta	Groups	Control	-,0415	,1762	-,0900	,6900
		Pneumonia	,0323	,1389	,0500	,5200
		COVID-19	,0075	,2193	-,0050	,6700
IL-17_delta	Groups	Control	,1750	1,9276	,3500	4,2000
		Pneumonia	,0714	1,6469	,5000	4,3000
		COVID-19	-,0333	2,8290	1,6000	4,9000
IL-18_delta	Groups	Control	-5,5000	91,2159	-9,5000	498,0000
		Pneumonia	-4,8333	100,3418	-6,0000	410,0000
		COVID-19	-95,0000	203,4939	-51,0000	670,0000

MIP-1 alpha_delta	Groups	Control	12,4167	24,9780	16,0000	75,0000
		Pneumonia	-13,1250	30,3268	-14,5000	118,0000
		COVID-19	4,0000	23,8956	9,0000	47,0000
MIP-1 beta_delta	Groups	Control	-15,7500	69,4258	-28,5000	239,0000
		Pneumonia	-11,0000	84,7899	-9,0000	340,0000
		COVID-19	-53,8000	97,0358	-58,0000	285,0000
MMP-3_delta	Groups	Control	-,1044	,9194	-,1000	5,0000
		Pneumonia	-,3162	1,7095	-,5000	7,0000
		COVID-19	-,3875	,4883	-,3000	1,4000
MMP-9_delta	Groups	Control	-5,3684	19,8248	-5,0000	65,0000
		Pneumonia	3,2500	21,0285	9,0000	69,0000
		COVID-19	5,8333	18,8087	-1,5000	44,0000
MCP-1_delta	Groups	Control	-22,1000	81,6369	-6,0000	292,0000
		Pneumonia	-6,6818	163,8697	45,5000	540,0000
		COVID-19	-134,2000	561,4406	-175,0000	2120,0000
Myoglobin_delta	Groups	Control	1,8619	13,3963	-1,0000	59,0000
		Pneumonia	,3571	10,9376	1,0000	42,0000
		COVID-19	,2500	3,2016	-1,0000	7,0000
PAI-1_delta	Groups	Control	-8,5652	39,7479	1,0000	150,0000
		Pneumonia	32,7391	63,1724	24,0000	267,0000
		COVID-19	-19,1000	49,7381	-27,5000	166,0000
PARC_delta	Groups	Control	-9,2400	62,2356	4,0000	326,0000

		Pneumonia	30,5652	119,0633	6,0000	665,0000
		COVID-19	-1,3000	22,4155	-11,0000	67,0000
SAP_delta	Groups	Control	-,4522	2,9285	-,7000	14,0000
		Pneumonia	-,6048	3,2358	,3000	14,0000
		COVID-19	-1,4444	2,6977	-2,0000	7,0000
SCF_delta	Groups	Control	-6,1522	84,7455	26,0000	328,0000
		Pneumonia	-25,6053	129,7365	36,0000	478,0000
		COVID-19	-68,5000	145,2810	-26,0000	359,0000
RANTES_delta	Groups	Control	-2,6043	9,5327	-2,0000	41,0000
		Pneumonia	-1,6186	6,5778	-1,0000	30,8000
		COVID-19	,2000	3,8178	-1,0000	10,3000
TBG_delta	Groups	Control	,0455	6,9862	2,0000	32,0000
		Pneumonia	-1,0909	8,0054	,0000	30,0000
		COVID-19	-5,7778	5,9954	-5,0000	20,0000
TIMP-1_delta	Groups	Control	-6,2308	47,2130	3,0000	235,0000
		Pneumonia	18,5217	81,1440	14,0000	425,0000
		COVID-19	18,7000	153,8318	-23,5000	594,0000
TNF-alpha_delta	Groups	Control	1,0000	.	1,0000	,0000
		Pneumonia	33,5000	,0000	33,5000	,0000
		COVID-19	-30,5000	.	-30,5000	,0000
TNFR2_delta	Groups	Control	-4,6625	25,1806	-,4000	136,0000
		Pneumonia	1,5652	9,2826	3,0000	38,0000

		COVID-19	3,7800	24,3492	-2,6000	89,0000
VCAM-1_delta	Groups	Control	-1,3846	168,9885	2,0000	791,0000
		Pneumonia	75,4762	233,0278	13,0000	1080,0000
		COVID-19	-5,0000	138,3962	-20,0000	468,0000
VEGF_delta	Groups	Control	-1,0435	59,4623	6,0000	259,0000
		Pneumonia	29,0476	79,9015	8,0000	349,0000
		COVID-19	-24,0000	92,2870	-45,0000	351,0000
VDBP_delta	Groups	Control	-4,2000	38,7933	-8,0000	178,0000
		Pneumonia	2,2609	37,5018	3,0000	159,0000
		COVID-19	-8,6000	37,5949	-3,5000	127,0000
vWF_delta	Groups	Control	-13,1200	65,5968	-8,0000	272,0000
		Pneumonia	-21,2727	176,8785	8,5000	1021,0000
		COVID-19	-64,8000	130,1109	-50,0000	479,0000
AFP_delta	Groups	Control	-,0820	,7295	-,4000	1,4200
		Pneumonia	,1318	,8746	,7100	2,5200
		COVID-19	,7100	.	,7100	,0000
ANG-1_delta	Groups	Control	,3846	10,1354	,0000	47,0000
		Pneumonia	1,7762	11,6924	1,0000	56,0000
		COVID-19	-3,3889	4,7551	-2,0000	16,0000
AXL_delta	Groups	Control	,4114	1,0057	,2500	3,5000
		Pneumonia	-,1417	1,0647	-,2500	4,0000
		COVID-19	,0875	1,2100	,1000	4,2000

CA-125_delta	Groups	Control	-2,7500	7,0373	-2,0000	22,1500
		Pneumonia	-2,0275	12,0045	2,1000	47,0000
		COVID-19	1,8000	6,4285	2,4500	15,6000
CA-19-9_delta	Groups	Control	1,3955	6,9721	,0000	27,0000
		Pneumonia	-1,0912	8,1036	-1,0000	36,0000
		COVID-19	-1,6188	6,1709	-2,4250	21,0000
CA-9_delta	Groups	Control	,0151	,1026	-,0001	,5300
		Pneumonia	-,0053	,0356	,0100	,1200
		COVID-19	,0004	,0567	-,0235	,1600
CEA_delta	Groups	Control	-,2347	1,0656	-,1000	4,5100
		Pneumonia	-,1232	,5443	-,1000	2,5000
		COVID-19	,0350	,6395	-,1000	2,1000
HCC-4_delta	Groups	Control	,0318	,3969	,1000	1,6000
		Pneumonia	,0700	,6219	-,1000	2,0000
		COVID-19	,1171	,1472	,1000	,4400
Decorin_delta	Groups	Control	,0517	,2130	,1000	,9000
		Pneumonia	-,0353	,3278	-,1000	1,4000
		COVID-19	,0143	,3237	-,2000	,8000
FAS_delta	Groups	Control	-1,2667	4,5906	-2,0000	20,6000
		Pneumonia	,1000	7,5247	-,5000	23,0000
		COVID-19	4,2700	16,2882	1,5000	63,0000
HGF_delta	Groups	Control	-,5304	1,8497	-,8000	8,0000

		Pneumonia	-2,8500	7,4527	-2,5000	36,0000
		COVID-19	-9,2556	29,6102	-3,0000	101,0000
hCG_delta	Groups	Control	-,3000	.	-,3000	,0000
		Pneumonia	-,0875	1,6555	,3500	3,8500
		COVID-19	,6667	2,0306	-,3000	3,7000
IL-18bp_delta	Groups	Control	,0000	1,1662	-,1000	4,0000
		Pneumonia	-,6923	3,9872	-1,0000	13,0000
		COVID-19	-,1900	2,6510	-1,0000	8,0000
MMP-1_delta	Groups	Control	-,6042	2,8472	,0500	14,0000
		Pneumonia	-,6000	10,1490	1,0000	52,0000
		COVID-19	-,4100	5,0479	-1,5000	18,0000
MMP-7_delta	Groups	Control	,0840	1,2912	,2000	7,0000
		Pneumonia	-,0833	1,1346	-,1000	4,0000
		COVID-19	-,4286	3,2201	-,2000	10,4000
MMP-9, total_delta	Groups	Control	-69,7692	335,2541	-21,0000	1726,0000
		Pneumonia	-57,6957	204,1705	-56,0000	987,0000
		COVID-19	-269,2000	411,2982	-165,5000	1380,0000
NSE_delta	Groups	Control	,4152	1,3924	,0200	7,3100
		Pneumonia	-,0690	,3967	-,1000	1,5900
		COVID-19	-,1522	2,0987	-,3000	7,6100
PECAM-1_delta	Groups	Control	-,2917	7,7487	2,0000	35,0000
		Pneumonia	-2,3333	14,1751	2,0000	65,0000

		COVID-19	-1,8889	10,5883	-5,0000	32,0000
SP-D_delta	Groups	Control	-,2125	1,8319	-,1000	9,0000
		Pneumonia	-2,9476	7,3474	-1,0000	35,0000
		COVID-19	,5556	9,1667	-2,0000	30,0000
TRAIL-R3_delta	Groups	Control	,1261	,9117	,1000	3,7000
		Pneumonia	-,7667	2,6337	-1,0000	12,0000
		COVID-19	-,4222	,9378	-,6000	3,1000

Table 18. List of all biomarkers with their means of delta values (arterial minus venous), standard deviation and median.

Biomarkers	Groups	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
EN-RAGE	COVID-19 and Pneumonia	12.993	6.506	1.997	.046	.137
	COVID-19 and Control	21.296	6.391	3.332	.001	.003
	Pneumonia and Control	8.303	4.916	1.689	.091	.274
IgM	COVID-19 and Pneumonia	10.710	5.972	1.793	.073	.219
	COVID-19 and Control	15.468	6.013	2.573	.010	.030
	Pneumonia and Control	4.759	4.604	1.034	.301	.904
Il-1RA	COVID-19 and Pneumonia	8.629	6.266	1.377	.168	.505

	COVID-19 and Control	18.960	6.102	3.107	.002	.006
	Pneumonia and Control	10.331	4.827	2.140	.032	.097
Il-10	COVID-19 and Pneumonia	9.900	4.373	2.264	.024	.071
	COVID-19 and Control	1.750	4.958	.353	.724	1.000
	Pneumonia and Control	-8.150	4.373	-1.864	.062	.187
NSE	COVID-19 and Pneumonia	6.262	6.382	.981	.327	.980
	COVID-19 and Control	14.173	6.227	2.276	.023	.069
	Pneumonia and Control	7.911	4.742	1.668	.095	.286
PAI-1	COVID-19 and Pneumonia	16.241	6.177	2.629	.009	.026
	COVID-19 and Control	4.089	6.177	.662	.508	1.000
	Pneumonia and Control	-12.152	4.809	-2.527	.011	.034

Table 19. List of statistically significant biomarkers using Kruskal Wallis Test on delta values among three different groups

8. Publications / Acknowledgments

8.1. Publications

Distinct patterns of blood cytokines beyond a cytokine storm predict mortality in COVID-19; Herr C. et al.; Journal of Inflammation Research

8.2. Acknowledgments

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9. Curriculum vitae

For data protection reasons, the curriculum vitae will not be published in the electronic version of the dissertation.

Publications

Prevalence of acne and its impact on the quality of life in high school-aged adolescents in Yazd, Iran; Journal of Pakistan Association of Dermatologists 2013;23 (2):168-172.

Distinct patterns of blood cytokines beyond a cytokine storm predict mortality in COVID-19; Herr C. et al.; Journal of Inflammation Research. 2021 Sep 15;14:4651-4667.

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