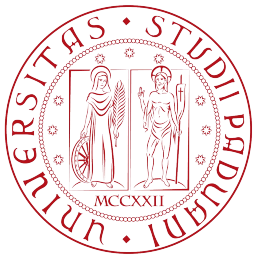

Università degli Studi di Padova

Corso di laurea magistrale in Medicina e Chirurgia

DIPARTIMENTO DI SCIENZE
CARDIO-TORACO-VASCOLARI E SANITÀ
PUBBLICA

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Tesi di Laurea

"PREDICTORS OF LUNG COMPLICATIONS AFTER COVID-19 PNEUMONIA"

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Contents

ABSTRACT

Background

Since December 2019, when COVID-19 emerged in Wuhan and rapidly spread worldwide, an overcrowding of health care facilities and limitations of medical resources have established. As a consequence of this, in order to improve the management of patients and optimize the use of resources, predictors of disease severity and lung complications after COVID-19 pneumonia urgently need to be found.

Aim of the study

This retrospective study aims to evaluate the significance of hematological values in patients hospitalized for COVID-19 pneumonia. The first goal is to investigate a possible association between serum values and the intensity of care that patients needed during hospitalization. The second goal is to explore the relationship between inflammatory markers (such as NLR, CRP, D-Dimer and eosinophils) and COVID-19 sequelae after discharge.

Materials and methods

In this study 327 patients, categorized in high intensity medical care group (HIMC, n=113) and low intensity medical care group (LIMC, n=214), are enrolled. For the whole population, clinical, radiological and demographics data are obtained at the hospital admission and at the first follow-up visit (3 months). A complete blood test in order to calculate biomarkers (NLR, LMR, CRP, Δ eosinophils) is also collected at admission and on discharge. Treatment during hospitalization is finally reported.

Results

In the univariate analysis age $\geq$ 62 years (p=0.002), a high degree of medical care (p=0.0001), NLR at admission $\geq$ 4,64 (p=0.02), neutrophils at admission $\geq$ 4.25 x$10^9/L$ (p=0.002), CRP at admission $\geq$ 59.5 (mg/dl) (p=0.007), ferritin at admission $\geq$ 589 (ng/ml) (p=0.04), Δ eosinophils $\geq$ 0.05 (p=0.002) and oncological diseases (p=0.04) are associated with persistent radiological abnormalities at follow-up. In multivariate analysis, age $\geq$ 62 years (p=0.03) and Δ eosinophils $\geq$ 0.05 (p=0.03) are two independent predictor factors of radiological lung sequelae in the whole population. Moreover, NLR at admission and Δ eosinophils positively correlate with alveolar score (r=0.30, p=0.002; r=0.20, p=0.04; respectively) and interstitial score (r=0.22, p=0.02; r=0.27, p=0.003 respectively) at first CT scan after discharge (3 months). Similarly, a positive correlation has been observed between lung ultrasound score at first follow-up, NLR and Δ eosinophils (r=0.13, p=0.02; r=0.14, p=0.02 respectively).

Conclusions

Based on our findings, NLR at baseline and Δ eosinophils could be potential predictors of radiological sequelae in CT scan, even though further studies are needed to investigate the role of blood values in post COVID sequelae.

RIASSUNTO

Background

A partire da dicembre 2019, quando il COVID-19 ha fatto per la prima volta comparsa a Wuhan e si è rapidamente diffuso in tutto il mondo, si è verificato un sovraffollamento delle strutture sanitarie e una limitazione delle risorse mediche. Di conseguenza, al fine di migliorare la gestione dei pazienti e ottimizzare l'uso delle risorse, è necessario trovare urgentemente dei predittori di gravità di malattia e di complicazioni polmonari successive alla polmonite da COVID-19.

Scopo dello studio

Questo studio retrospettivo mira a valutare il significato dei parametri ematologici nei pazienti ricoverati per polmonite da COVID-19. Il primo obiettivo è quello di indagare una possibile associazione tra i valori sierici e l'intensità delle cure di cui i pazienti necessitano durante il ricovero. Il secondo obiettivo è quello di esplorare la relazione tra i marcatori infiammatori (come NLR, PCR, D-Dimero e Δ eosinofili) e le sequele del COVID-19 dopo la dimissione.

Materiali e metodi

In questo studio sono stati arruolati 327 pazienti, suddivisi in base al livello di cure in gruppo ad alta intensità di cure (HIMC, n = 113) e gruppo a bassa intensità di cure (LIMC, n = 214). Per l'intera popolazione, i dati clinici, radiologici e demografici vengono raccolti il giorno del ricovero ospedaliero e alla prima visita di controllo (3 mesi). Un emocromo completo per calcolare i biomarcatori (NLR, LMR, PCR e Δ eosinofili) viene raccolto sia al momento del ricovero che il giorno della dimissione. Viene

inoltre riportata la terapia eseguita durante il ricovero.

Risultati

Nell' analisi univariata età ≥ 62 anni (p=0.002), un alto grado di assistenza medica (p=0.0001), NLR al ricovero ≥ 4.64 (p=0.02), neutrofili al ricovero ≥ 4.25 x$10^9/L$ (p=0.002), PCR al ricovero ≥ 59.5 (mg/dl) (p=0.007), ferritina al ricovero ≥ 589 (ng/ml) (p=0.04), Δ eosinofili ≥ 0.05 (p=0.002) e malattie oncologiche (p=0.04) sono associati alla persistenza di anomalie radiologiche al follow-up. Nell'analisi multivariata età ≥ 62 anni (p=0.03) e Δ eosinofili ≥ 0.05 (p=0.03) sono due predittori indipendenti di sequele radiologiche polmonari nell'intera popolazione di pazienti. Inoltre, NLR al ricovero e Δ eosinofili correlano positivamente con lo score alveolare ($r = 0.30$, $p = 0.002$; $r = 0.20$, $p = 0.04$; rispettivamente) e lo score interstiziale ($r = 0.22$, $p = 0.02$; $r = 0.27$, $p = 0.003$ rispettivamente) alla prima scansione TC al follow-up (3 mesi). Similmente è stata osservata una correlazione positiva tra score ecografico al primo follow-up, NLR e Δ eosinofili ($r = 0.13$, $p = 0.02$; $r = 0.14$, $p = 0.02$ rispettivamente).

Conclusioni

Sulla base dei nostri risultati, NLR al ricovero e Δ eosinofili potrebbero essere potenziali predittori di sequele radiologiche alla TC, anche se sono necessari ulteriori studi per indagare più approfonditamente il ruolo dei parametri ematologici nelle sequele post COVID.

INTRODUCTION

1.1 Coronaviruses

Coronaviruses are a varied group of viruses, characterized by the possession of a single-strand positive-sense RNA genome [1].
The name coronavirus is evocative and refers to the fact that virus particles have peculiar spikes, which give the virions the look of a crown (or corona)[2].
This group of enveloped viruses belongs to the order of Nidovirales [3] and to the family of Coronaviridae and can infect many different species of animals.
Coronaviruses are able to cause a heterogeneous spectrum of manifestation in humans, such as neurologic, hepatic and enteric diseases [4]. However, the main symptoms of coronavirues are attribuitable to the infection of the respiratory tract.
Known to date, in literature six coronavirus species that could affect human people have been described, and in particular four of them (229E, OC43, NL63, HKU1) are usually linked to cold symptoms and the other two (SARS-CoV and MERS-CoV) may hesitate in fatal illness [5].

1.1.1 SARS-CoV

Outbreaking in February 2002 in Chinese province of Guandong, Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) had been the causative agent of an epidemy of atypical pneumonia, which successively spread mainly all over the country, but also overseas.
This virus is characterized by an elevated rate of mortality, and in fact, by July 2003, the WHO declared 8437 cases of SARS worldwide and attributed 813 deaths to this disease [6].

1.1.2 MERS-CoV

Middle East Respiratory Syndrome coronavirus (MERS-CoV) was found for the first time in 2012, in Saudi Arabia, in the lung tissue of a 60-year-old man who was affected by a severe acute pneumonia [7]. Subsequently, this virus soon spread over other countries in the Middle East and the last report from the WHO, dated 31 January 2020, showed a total number of 2519 cases, with 866 deaths, which denotes an extremely high mortality rate (34,4%) [8].

1.1.3 SARS-CoV-2

Between the end of December 2019 and the approach of 2020, several cases of an unknown origin pneumonia broke out in the city of Wuhan, China [9].
The first analysis, made on bronchoalveolar lavage of the patients, have shown the presence of a new viral agent, never seen before. After genome sequences assays, virologists have classified this new infective agent as a member of coronaviruses, and designated it temporary as 2019-nCoV [10].
Subsequently, further studies demonstrated that 2019-nCoV has

the 80% of genome homology with SARS-CoV and the 50% with MERS-CoV [11]. As a consequence of this, 2019-nCov has been collocated in the subgenre of *betacoronavirus* and renamed as "SARS-CoV-2". Moreover, SARS-CoV-2 has also demonstrated a homology of 96% with bat coronavirus BatCov RaTG1. This fact enhances the theory according to which SARS-CoV-2 probably originates from bats, and that these animals are the natural reservoir of the virus [12].

Unlike the other zoonotic viruses MERS-CoV and SARS-CoV, which usually cause severe respiratory illness, SARS-CoV-2 has a lover mortality rate, but, on the other hand, it shows a higher transmissibility [13]. This is the main reason why SARS-CoV-2 has rapidly spread from China to the other countries, rising a global epidemic which has sharply surpassed SARS-CoV and MERS-CoV for number of infected people [5]. (Figure 1)

	Coronavirus		
	SARS-CoV-2	*SARS-CoV*	*MERS-CoV*
Epidemiology			
Outbreak beginning date	December 2019	November 2002	April 2012
Location of the first case	Wuhan, China	Guangdong, China	Saudi Arabia
Confirmed cases	595.800 (Mar 27, 2020)	8096	2519 (From 2012 until January 31, 2020)
Mortality	27.324 (%)	744 (10%)	866 (34.4%)

Figure 1.1: *Characteristics of patients with SARS-CoV-2, SARS-CoV, and MERS-CoV,* adapted from: Rabaan et al.

1.2 COVID-19

1.2.1 Infection and receptor binding

In order to enter host cells, SARS-CoV-2 takes advantage of the interaction between its spike protein (named also "S protein") and ACE2 receptor.(Figure 2)

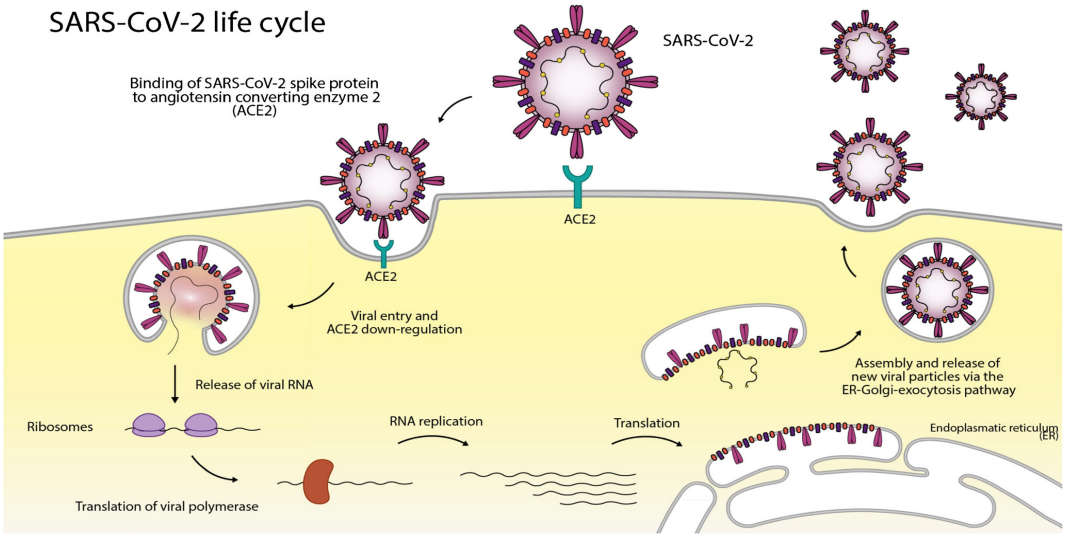


Figure 1.2: *SARS-CoV-2 life cycle: from binding to ACE2 receptor to shedding,* adapted from: Beyerstedt et al.

With the aim of binding ACE2, S protein needs the action of a host cells' surface protease, named furin, which cleaves S protein in the site S1/S2 [14]. This cleavage results in the formation of two distinct subunits, S1 and S2. S1's main role, during the infection, is to achieve the binding between the virus and ACE2 receptor [15].

After the cleavage by furin, S1 subunit binds to ACE2, and this process is also mediated by a host cell's tripsin-like transmembrane serin protease, known as TMPRSS2. (Figure 3)

In addition, it has been proven that in cells which don't express TMPRSS2 on the membrane, there is another endosomial ubiquitary protease, L catepsin, which processes S protein and allows the entrance of SARS-CoV-2 in host cells. This last statement should be remembered, because it represents another way for the virus to infect cells [16].

Besides S1, S2 subunit also plays an important role, because it permits the fusion between virus and cell's membrane, which is

essential for the infection process.

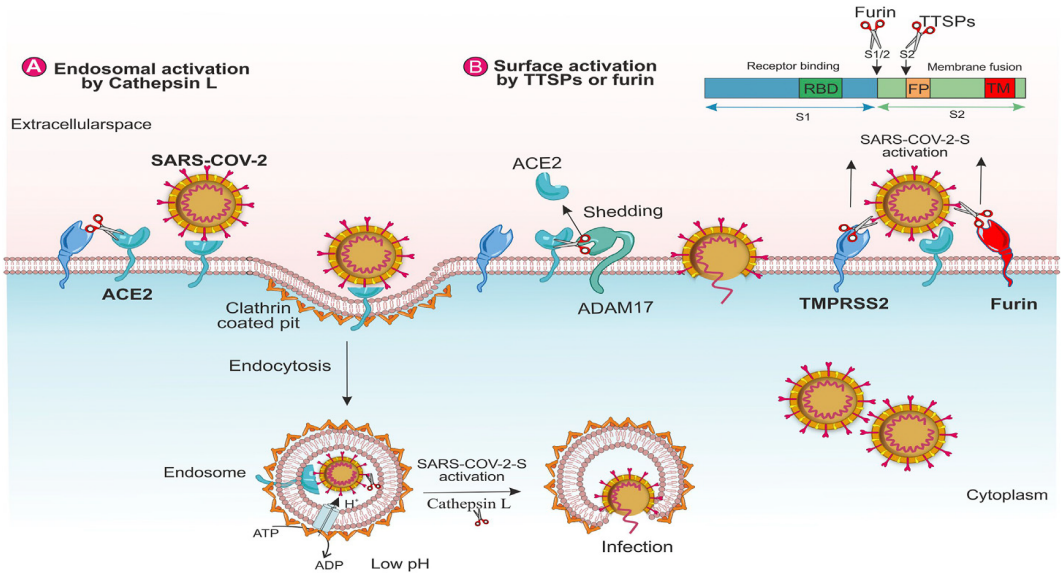


Figure 1.3: *The role of human host proteases on SARS-CoV-2 entry. Virus entry through (A) endosomal pathway and (B) TMPRSS2 and furin,* adapted from: Saadat et al.

1.2.1.1 Angiotensin Converting Enzyme (ACE2)

Angiotensin Converting Enzyme 2 (ACE2) is a receptor expressed in many different tissues, among which small intestine, kidney, heart muscle, colon, epithelial respiratory cells and type II alveolar cells (AT2) [17]. In particular, the expression of ACE2 in the alveolar cells has been emphasized after the outbreak of SARS-CoV-2 epidemy, in consequence of the primary role of this receptor in the pathogenesis of COVID-19 pneumonia. Indeed, ACE2 represents the entrance door for SARS-CoV-2 in host cells and, furthermore, it has been evidenced that the infection, by increasing inflammatory cytokines (first of all IFNs [18]), can enhances the expression of ACE2 and potentiates the infection itself [19].

1.2.2 Trasmission

Essentially, SARS-CoV-2 can be transmitted by three different ways: droplets, aerosol and contact with contaminated surfaces. However, according to literature, droplets expelled during coughing, sneezing and head-to-head talking, represent the main modality of SARS-CoV-2 transmission [20]. (Figure 4)
Particles have usually a diameter between 5 and 10μm, and, for this reason, they tend to fall to the ground within 100-150cm from the source [16]. As a consequence of this, it has been proved that social distancing and mask wearing are the best ways to prevent the infection.
Moreover, SARS-CoV-2 can be airborne spread through the aerosol (diameter <5 μm) steadily produced while breathing [21]. According to this hypothesis, a recent study found out that SARS-CoV-2 aerosol remains viable in the air for a duration of at least 3h, and, therefore, it can infect the human host [22]. However, this source of contagion is mostly common in closed and poorly ventilated environments.
Furthermore, another possible way of transmission is represented by the contact with infected surfaces and fomits, followed by self-hand touching of eyes and mouth. In this contest, the variability of persistence of SARS-CoV-2 on surfaces depends on the characteristics of the specific material of which the surface is made [23]. Moreover, SARS-CoV-2 has also been detected in faces and body fluids, such as urine, but there is still no evidence of the transmission through these biological materials [24].
An important factor related to the transmission of SARS-CoV-2 is the viral load, which consists in the quantity of virus particles in the biological materials of the host. High levels of viral load are linked to a greater contagiousness [25]. In relation to SARS-

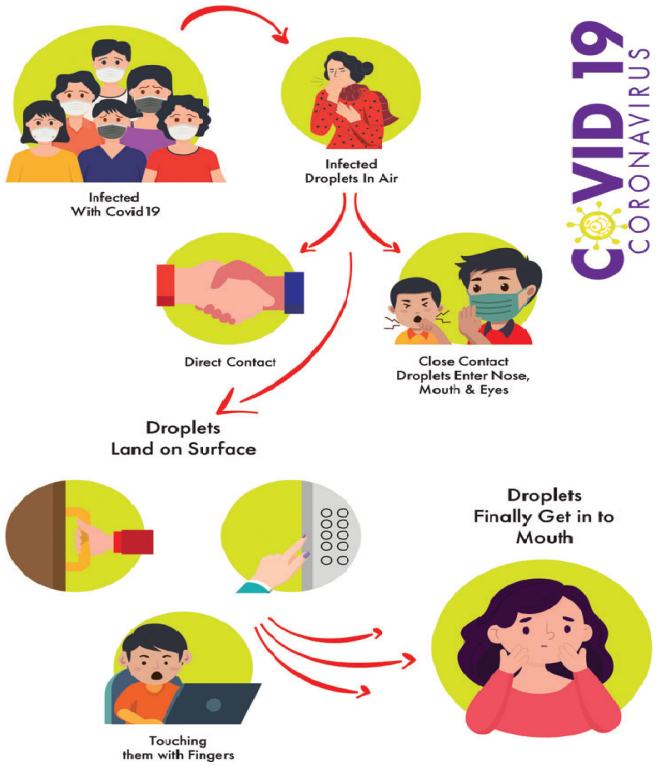


Figure 1.4: *Modes of transmission,* adapted from: Umakanthan et al.

CoV-2, viral load depends on the severity of illness and on the phase of disease.

Furthermore, it is important to remember that patients infected with SARS-CoV-2 may also spread virus during the incubation period, which has an average duration of 4-5 days [26, 27]. This aspect represents one of the main reasons behind the large diffusion of COVID-19, because asymptomatic people are difficult to trace and can shed virus without the knowledge of being infective.

1.2.2.1 R0 Index

An important parameter in the transmission dynamics of SARS-CoV-2 is the reproductive number, also known as R0. R0 index consists in the average number of novel cases generated by a single infected host, in a condition where no containment measures

are applied [16]. If health-care restriction measures are adopted, instead of R0, Rt index is used. The latter differs from the first because it is time-dependent [28].
R0 is crucial to assess if virus spreading is well controlled or not. As a consequence of this:

- if R0 <1, it means that an epidemic is well controlled;
- if R0 >1, it means that virus shedding is increasing and the epidemic is still rising.

In the first COVID-19 reports from China, it has been estimated a R0 between 2,2 and 2,7, which was related to a doubling of the infected people every 6-7 days [29].

1.2.3 Immunity

In response to SARS-CoV-2, both humoral and cellular immunity are involved.

1.2.3.1 Humoral immunity

By analyzing plasma samples belonging to COVID-19 patients, it has been documented the presence of IgA, IgM, and IgG class antibodies (Abs) against several SARS-CoV-2 antigens, among which S protein and N protein. In particular, S protein's RBD (Receptor Binding Domain) is the main target of humoral immunity. About this, recent studies found out that more than 90% of SARS-CoV-2 neutralizing antibodies are accounted for by RBD-directed Abs [30].
Similar to other infections, in COVID-19 the kinetics of neutralizing Abs depends on the specific antibody class which is considered. As a consequence of this, it has been observed that:

- IgA Abs begin to be detected during the first week and peak up to the end of the third week;
- IgM Abs reach high titres among 10-12 days and start to decrease after 18 days from the onset of symptoms;
- IgG Abs titres rise during the first three weeks after symptoms onset, and begin to drop by 8 weeks [31].

Besides, it has been shown that in subjects with mild disease IgG titres after illness decrease more rapidly in comparison to patients with severe or symptomatic COVID-19 [16]. However, the protective efficacy over time of specific Abs after the infection need to be investigated with further studies [32].

1.2.3.2 Cellular immunity

In response to COVID-19 an important role is also played by cellular immunity. In particular:

- Lymphocytes T CD4+ are part of cytotoxic TH1 response, characterized by the production of IFN-γ. This immunity cells response is mainly directed against spike protein, but also against other structural proteins, like N protein and S protein. However, too non-structural elements like nsp3, nsp4 and ORF8 are targeted by CD4+ lymphocytes;
- Lymphocytes T CD8+ are also skewed to TH1 response, particularly through the production of IFN-γ and TNF-α [31].

Keeping this in mind, recent studies found out that patients with a deficit of production of IFNs are more likely to develop severe forms of COVID-19 and a similar situation can be observed in subjects with lymphopenia [33, 34].

Despite this, it has been demonstrated that in people with severe forms of pneumonia, an increase of follicular T helper (THf) and TH17 lymphocytes can be found, and, in particular, the latter are linked to the excess of inflammation responsible for worse COVID-19 disease [16].

1.2.4 Pathogenesis

Nowadays, after more than two years of epidemic, the pathogenesis behind COVID-19 is not yet well understood. However, up to the actual literature, two successive phases have been described in COVID-19: infectious phase and inflammatory phase.

1.2.4.1 Infectious phase

First of all, SARS-CoV-2 binds to ACE-2 receptor on the epithelial cells of the upper respiratory tract and starts to replicate. Then the virus migrates down to the airways and enters into the alveolar epithelial cells, especially in type II pneumocytes, which have a great tropism for SARS-CoV-2 [35].

1.2.4.2 Inflammatory phase

As a result of the infection, SARS-CoV-2 interferes with the production of surfactant and causes alveoli collapse. Furthermore, the replication of virus is associated to the damage of pneumocytes and to the release of IFNs and IL-6. The cellular damage is followed by the activation of alveolar macrophages, which release inflammatory cytokines, including IL-6, IL-8 and TNFα. These mediators boost inflammatory response through the recruitment of neutrophils and macrophages, which amplify lung damage and causes the massive release of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-2, IL-6, IL-7, TNF-α, etc.), known as “cytokine storm”

[16, 36].(Figure 5) This loop of events hesitates in the development of severe pneumonia, and at worst into an acute respiratory distress syndrome (ARDS).

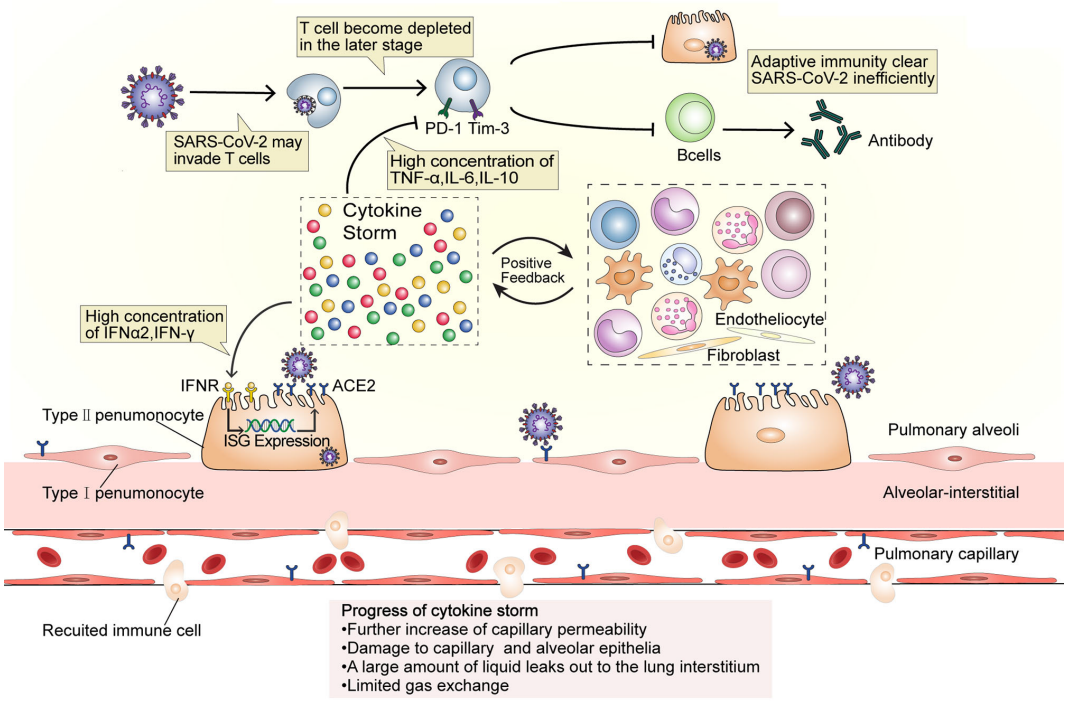


Figure 1.5: *Dysfunctional immune response induces cytokine storms,* adapted from: Chen et al.

In order to inhibit the excess of TH1 pro-inflammatory cytokines, TH2-response-shifted lymphocytes release anti-inflammatory mediators, such as IL-4 and IL-10. However, this attempt to shut down the immune system is not strong enough to balance the hyperinflammation, which plays a primary role in the pathogenesis of COVID-19 pneumonia [16].

Hyperinflammation is also followed by the development of coagulopathy, which is one of the main features of COVID-19 disease. In particular, the alterations of coagulation in COVID-19 pneu-

monia are attributable to:

- Downregulation of anticoagulant factors, first of all antithrombin III;
- Upregulated expression of tissue factor in macrophages, which starts the activation of coagulation and the generation of thrombin [37].

As a consequence of these variations, patients with COVID-19 develop a state of hypercoagulability which is linked to multiple thrombotic events and disseminated intravascular coagulation (DIC) [38].

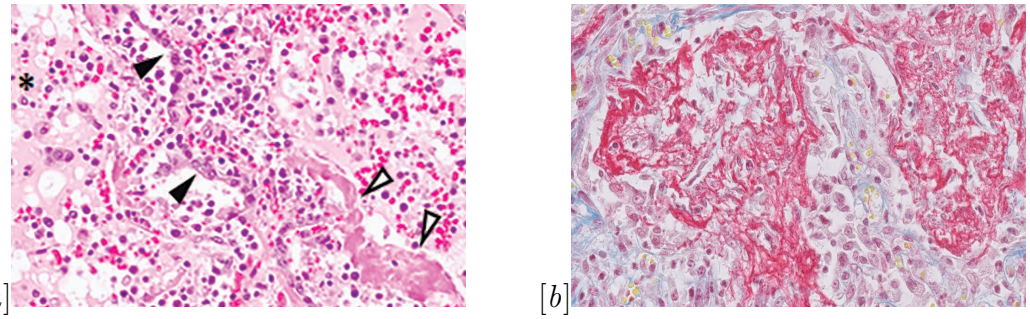
1.2.5 Histology

In patients with COVID-19 the major histopathological alterations can be found in the lungs.
Notably, during the first "exudative phase" it has been described the presence of bilateral diffuse alveolar damage (DAD), characterized by the formation of hyaline membranes (Figure 6a), interstitial and intra-alveolar edema and fibrin deposits [39] (Figure 6b). These pathological features interfere with gas exchange function of lungs and drive to the development of respiratory failure and hypoxia.
Furthermore, an important desquamation of bronchiole (Figure 7a) and alveolar epithelium (Figure 7b) have also been found. Through the analysis of autoptic lung samples from infected patients, several modifications of the alveolar architecture have been revealed. In particular, type I pneumocytes necrosis and type II pneumocytes atypical hyperplasia (Figure 8a) have often been detected [40]. These histological features are accompanied by an abundant interstitial and alveolar inflammatory infiltration, com-

posed mainly of macrophages (Figure 8b), lymphocytes (Figure 9a) and neutrophils (Figure 9b).

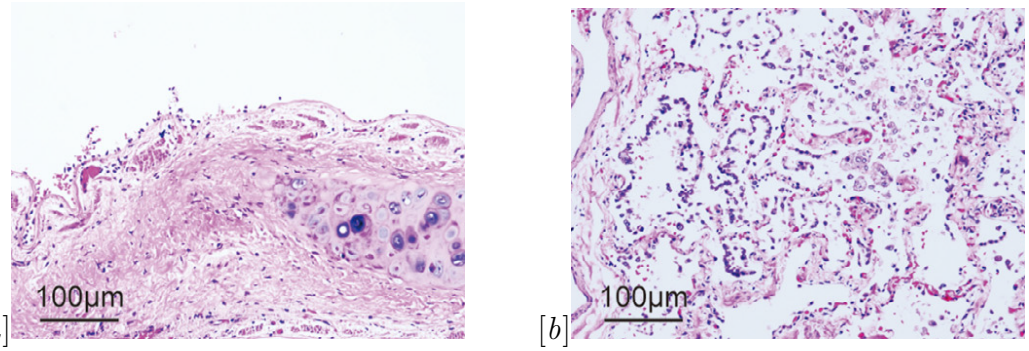
In patients with COVID-19 interstitial pneumonia, damages of endothelial cells of capillaries, venules and arteries have been also evidenced. Blood vessels' damage, together with hyperinflammation, is one of the main causes of COVID-19 coagulopathy, which is linked with the tendency of pulmonary thromboembolism (PTE) typical of this disease [41] (Figure 10a).

Following the first exudative phase, it has been described a "proliferative phase", which is featured by an exacerbated fibroblast and myofibroblast proliferation. This phase switch could cause an acute fibrinous organizing pneumonia (Figure 10b) with subsequent extracellular matrix deposition, which hesitates in parenchymal remodeling and pulmonary fibrosis [42].



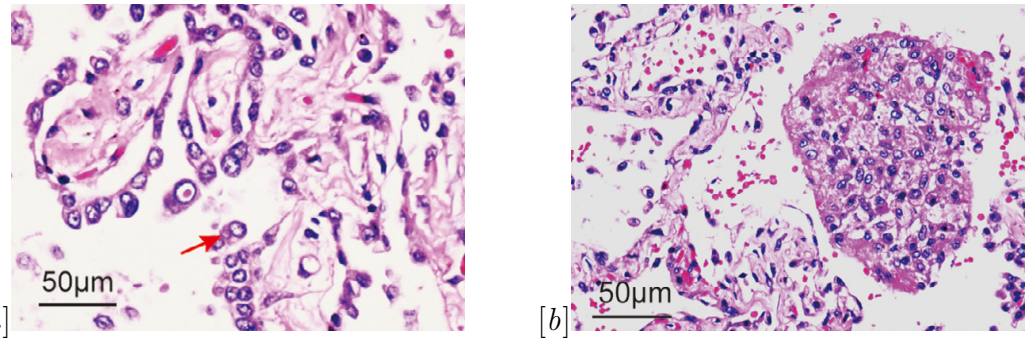
[a] [b]

Figure 1.6: *[a], Lung with diffuse alveolar damage featuring hyaline membranes (white arrowheads) and intra-alveolar edema (*),* adapted from: Yan et al.
[b], Alveolar spaces are filled with fibrin, stained in red, adapted from: LE van Eijk et al.



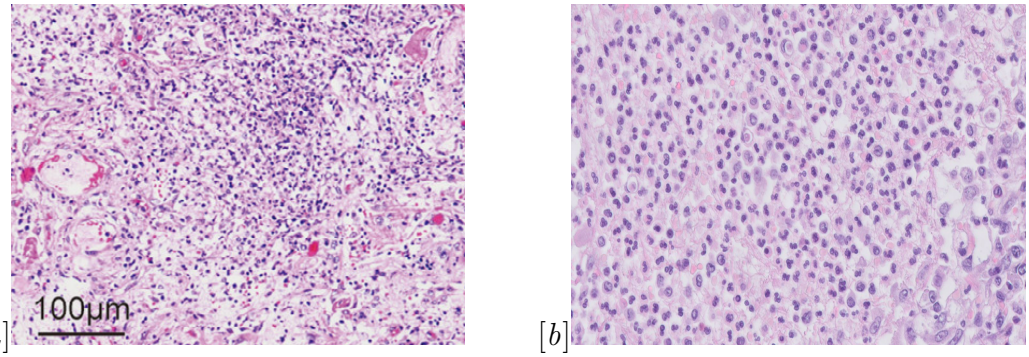
[a] [b]

Figure 1.7: *[a], Intensive sloughing of bronchiole epithelial cells,* adapted from: C.Wang et al.
[b], Desquamated swollen and degenerated alveolar cells in alveoli, adapted from: C.Wang et al.



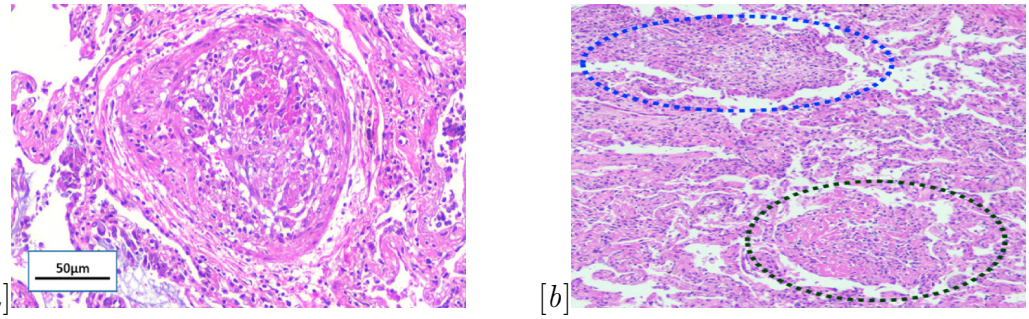
[a] [b]

Figure 1.8: *[a], Type II pneumocyte proliferation with atypical changes,* adapted from: C.Wang et al.
[b], Aggregation of macrophages in alveolI, adapted from: C.Wang et al.



[a] [b]

Figure 1.9: *[a], Pulmonary consolidation with infiltration of lymphocytes,* adapted from: C.Wang et al.
[b], Diffuse neutrophilic infiltrate in the alveolar spaces, adapted from: C.Wang et al.



[a] [b]

Figure 1.10: *[a], Pulmonary intravascular thrombotic events* , adapted from: C.Wang et al.
[b], Acute fibrinous organizing pneumonia (dark blue circle) and organizing pneumonia (dark green circle), adapted from: C.Wang et al.

1.2.6 Clinical features

As for clinical features, COVID-19 patients can be asymptomatic or symptomatic.

1.2.6.1 Asymptomatic infection

The expression "asymptomatic infection" refers to the detection of virus particles, through specific diagnostics assays, in patients with no typical clinical sign or symptoms, and no apparent abnormalities in imaging [43].
This is one of the most common clinical presentations of SARS-CoV-2 infection. Regarding this, an analysis of 565 infected patients from Wuhan, dated February 2020, showed an incidence of asymptomatics of about 30,8% [44]. However, this percentage is probably underestimated, in fact, more recent studies found out that the asymptomatic infections could stands between 43 and 77% [16].
Although, about the asymptomatics the most important assay to underline is that, in the same way as the symptomatics, they too are contagious.

1.2.6.2 Symptomatic infection

COVID-19 has a large spectrum of clinical presentations and the most characteristics symptoms are those about the respiratory tract. However, current literature has shown that COVID-19 is a multisystemic disease which can also affect other organs and systems.

1.2.6.2.1 Respiratory symptoms

First of all, COVID-19 is a respiratory disease. One of the first studies from Wuhan evidenced that the most common symptom is fever (85.3%), followed by dry cough (52.6%), fatigue (51.7%), anorexia (43.1%), dyspnea (44.8%), and chest discomfort (43.1%). Other less common respiratory symptoms as nasal obstruction (5.2%) and hemoptysis (0.9%) can also be detected [45].
As a consequence of the variety of clinical presentations, on the 19 October 2021, the WHO classified COVID-19 disease into four different stages of severity, on the basis of respiratory symptoms. In particular, it may be identified:

- Mild disease: patients who have experienced signs and symptoms of COVID-19, but have no abnormalities at chest imaging, dyspnea or shortness of breath;
- Moderate disease: patients with an oxygen saturation (SpO2) of 94% on room air and in whom evidence of lower respiratory disease during imaging or clinical assessment can be detected;
- Severe disease: patients in whom can be registered a respiratory rate >30 breaths/min, a SpO2 <94% on room air, a ratio of pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mm Hg, or lung infiltrates >50%;

- Critical disease: patients who are affected by respiratory failure, MOD (multi organ dysfunction) or septic shock.

Looking at the percentage, mild and moderate diseases represent the 80% of total cases of symptomatic COVID-19, severe disease can be found in about 15% of patients and critical illness stands at 5%.

1.2.6.2.2 Neurological symptoms

Besides the respiratory symptoms, in patients with COVID-19, a large variety of manifestations could be found. Neurological symptoms as anosmia (5,1%) and ageusia (5,6%) have often been detected. These features are quite specific of SARS-CoV-2 infection and they are probably due to the direct damage of the virus on the olfactory and gustatory receptors. Especially in paucisymptomatic patients, in a quite percentage of cases, anosmia and ageusia represent the first or the only symptoms of COVID-19 disease [46].

1.2.6.2.3 Gastrointestinal symptoms

COVID-19 is also linked to gastrointestinal manifestations, as nausea or vomiting (7%), diarrhea (9%) and abdominal pain (3%). The pathophisiology of these symptoms is probably related to:

- virus direct cellular damage, which is allowed by the expression of ACE2 in the gastrointestinal tract;

- inflammation-mediated tissue damage, which is provided by the presence of infiltrating plasma cells and lymphocytes in the lamina propria of stomach and duodenum [47].

1.2.6.2.4 Cardiovascular symptoms

Moreover, in severe form of COVID-19 also cardiovascular damages like arrhythmias (17%), myocardial injuries (7%) and shock (9%) have been detected [16].

1.2.6.2.5 Less common symptoms

Other less common sites damaged in the course of COVID-19 are kidney, hepatobiliary tract, ocular system, urinary system and skin [48].

1.2.7 Imaging

Imaging tools as chest X-ray (CXR), chest CT scan and lung ultrasound (LUS) play a fundamental role in the process of diagnosis of COVID-19 disease.

1.2.7.1 Chest X-Ray (CXR)

In comparison with CT, CXR is less sensitive, with a reported baseline sensitivity of 69%. However, this imaging tool can be helpful to identify COVID-19 lung patterns.
The most common findings in a COVID-19 CXR include bilateral ground glass opacities (GGO) and lung consolidations (Figure 11). Furthermore, reticular opacities (Figure 12a) accompanying regions of ground glass attenuation can be appreciated. Other features that may be identified are bilateral multifocal air-space diseases, patchy or confluent peripheric lung opacities (Figure 12b) and pulmonary nodules [49, 50].

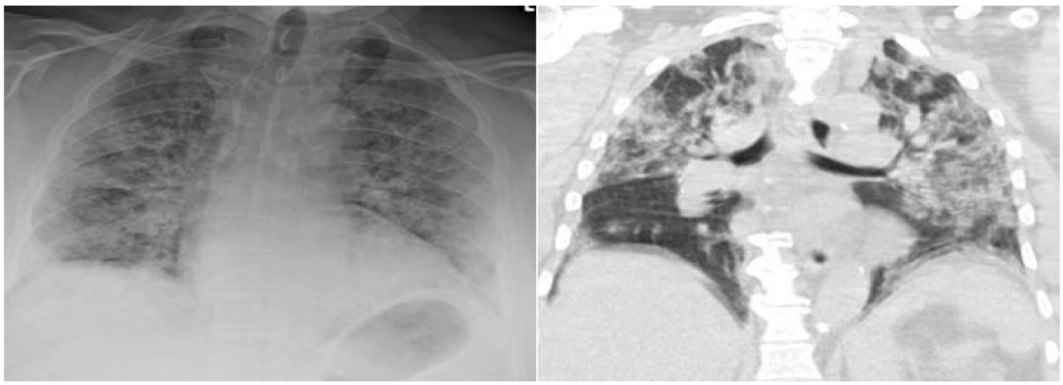
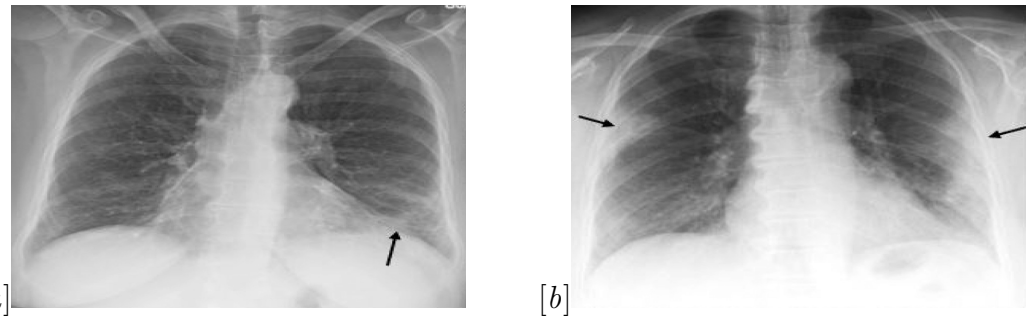


Figure 1.11:
CXR (left) and subsequent coronal image from chest CT (right) performed in a patient with COVID-19 and diffuse ground glass and consolidative opacities throughout both lungs, adapted from: A. Jacobi et al.



[a] [b]

Figure 1.12: *[a], CXR (left) with reticular and hazy left lower lobe opacities (black arrow) in a patient with COVID-19,* adapted from: A. Jacobi et al.
[b],Bilateral peripheral lungs opacities (black arrows), adapted from: A. Jacobi et al.

1.2.7.2 Chest CT scan

Chest CT scan can accurately evaluate the type and the extension of lung lesions, due to its high sensitivity (98%).
The predominant features appreciable in a chest CT scan of COVID-19 disease are bilateral GGO and consolidation in posterior and peripherial lungs. GGO (Figure 13a) are hazy areas with slightly increased density, which may be caused by partial displacement of air due to partial filling of airspaces or interstitial thickening. Consolidation (Figure 13b) can be defined as the replacement of alveolar air with pathological fluids, cells, or tissues, which results in an enhancement in pulmonary parenchymal density.

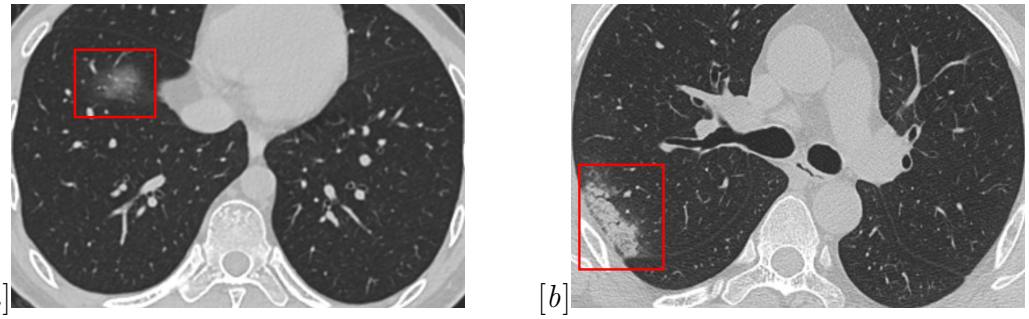


Figure 1.13: *[a], CT scan shows a pure ground glass opacity in the right lower lobe (red frame),* adapted from: Ye et al.
[b], CT scan shows consolidation in the right lobe subpleural area (red frame), adapted from: Ye et al.

Multifocal or segmental consolidation, with a main distribution along bronchovascular bundles or in subpleural areas, can be usually detected in COVID-19 patients. According to literature, this feature is the reflection of cellular fibromyxoid exudates in alveoli. Another common finding in COVID-19 lung is reticular pattern (Figure 14 a). This consists in the thickening of pulmonary interstitial structures, which manifests as a collection of innumerable small linear opacities on CT images. The formation of this pattern might be associated with interstitial lymphocyte infiltration, causing interlobular septal thickening.
A specific feature detected in COVID-19's lung is the "crazy paving pattern" (Figure 14b), which consists in thickened interlobular septa and intralobular lines with superimposition on a GGO background, resembling irregular paving stones. This sign is less frequent than consolidation and GGO and it may result from the alveolar edema and interstitial inflammation of acute lung injury.

Also, air bronchogram (Figure 15a), which consists in a pattern of air-filled bronchi on a background of opaque airless lung, has been detected in COVID-19's CT scan. This sign can be accompanied by airway changes like bronchiectasis and bronchial wall

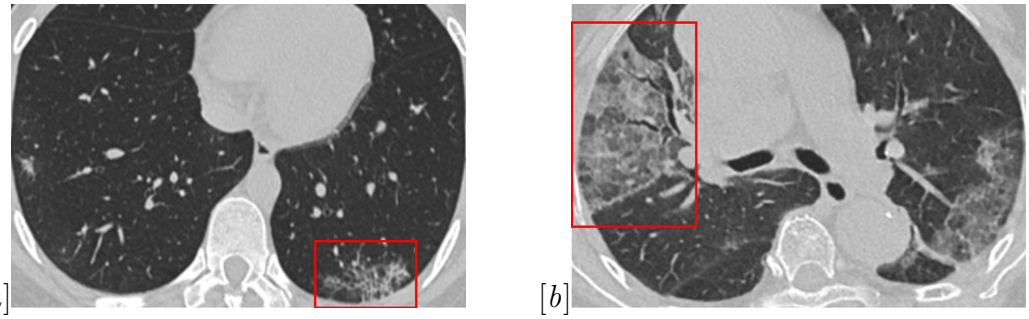


Figure 1.14: *[a], CT scan shows slight reticular pattern in the left lower lobe and subpleural area (red frame),* adapted from: Ye et al.
[b], CT scan shows reticular pattern superimposed on the background of GGO, resembling the sign of crazy paving stones in the right middle lobe (red frame), adapted from: Ye et al.

thickening (Figure 15b). The pathogenesis of these features can be identified in the inflammatory damage of bronchial wall and in the bronchial obstruction, which result in the destruction of bronchial wall structure, proliferation of fibrous tissue, fibrosis and tractive bronchiectasis.

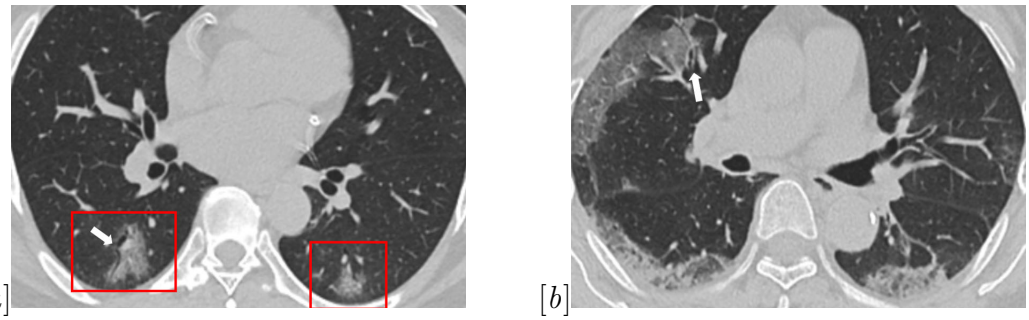


Figure 1.15: *[a], CT scan shows bilateral GGO in the lower lobe (red frames) and air bronchogram (white arrow) in the left subpleural area,* adapted from: Ye et al.
[b], CT scan shows reticular pattern in the subpleural areas of the bilateral lower lobe, GGO, and bronchial wall thickening (white arrow) in the right middle lobe, adapted from: Ye et al.

In addiction, pleural changes such as pleural thickening (Figure 16a) and pleural effusion have also been detected. Furthermore, in some patients subpleural curvilinear line can be seen (Figure 16b). This is defined as a thin curvilinear opacity with 1-3mm thickness, lying less than 1 cm from and parallel to the pleural surface.

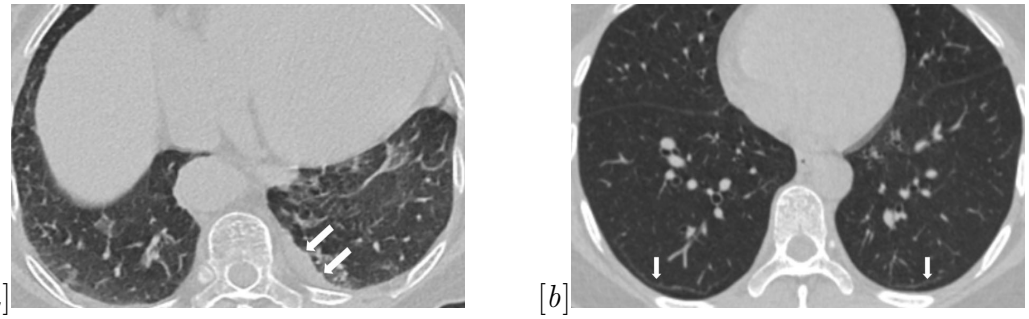


Figure 1.16: *[a], CT scan shows left pleural thickening (white arrows),* adapted from: Ye et al.
[b], CT scan shows subpleural lines (white arrows) in bilateral lower lobes, adapted from: Ye et al.

Other features which have been identified in COVID-19 lung are fibrosis (Figure 17a), vascular enlargement (Figure 17b), nodules, lymphadenopathy and "Halo sign". Halo sign (Figure 18a) refers to nodules or masses surrounded by ground glass and it might be related to viral infections and organizing pneumonia. It has been also detected a "reversed Halo sign" or "atoll sign" (Figure 18b), which consists in a focal rounded GGO surrounded by more or less complete ring-like consolidation. This feature might be attributed to disease progression, making consolidation developed around GGO or lesion absorption leaving a decreased intensity in the center [51].

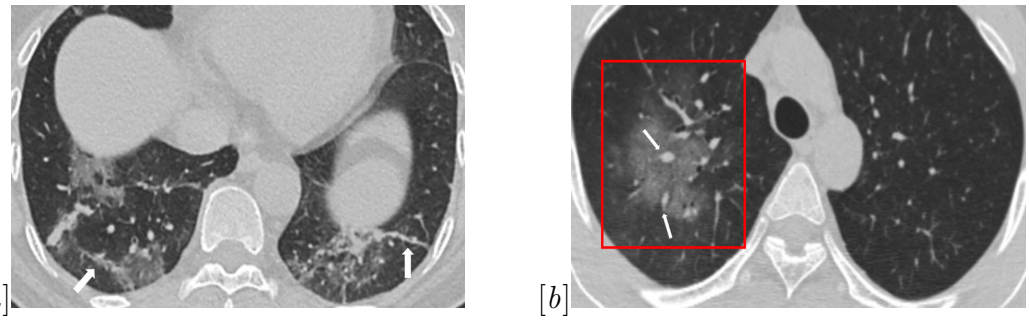


Figure 1.17: *[a], CT scan shows bilateral GGO and fibrous stripes (white arrows) in the left lower lobe,* adapted from: Ye et al.
[b], CT scan shows a large area of GGO (red frame) in the right upper lobe with multiple small vascular enlargement (white arrows), adapted from: Ye et al.

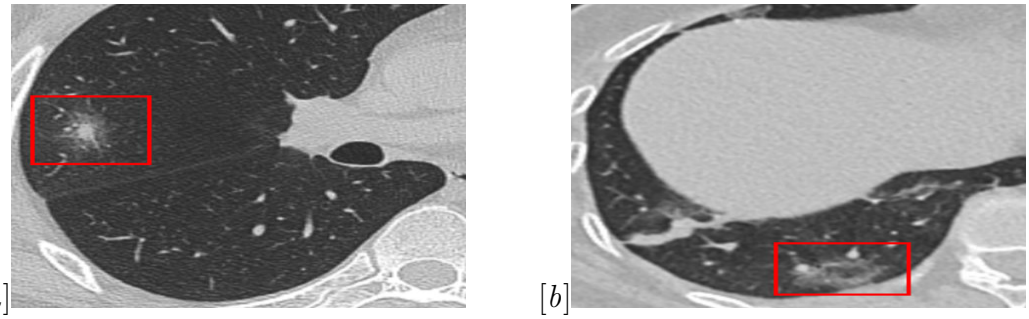


Figure 1.18: *[a], CT scan shows a solid nodule surrounded by a ground glass halo in the lateral segment of the right middle lobe (red frame),* adapted from: Ye et al. *[b], CT scan shows a reversed halo sign (red frame) in the posterior basal segment of the right lower lobe,* adapted from: Ye et al.

1.2.7.3 Lung Ultrasound (LUS)

Lung ultrasound (LUS) is an imaging tool which can be used in the diagnosis of COVID-19, with a sensitivity of 78%.
This technique is useful especially because it allows a rapid diagnosis and can be performed at any stage of disease and, most importantly, at the patient's bedside.
In the course of COVID-19 infections, through the use of LUS, features of interstitial pneumonia can be detected. In this context, peculiarly findings are irregular pleural line (Figure 19a), multifocal, discrete or confluent B lines (Figure 19b) and consolidations. Fluid in the pleural cavity is rare and can be found in small volumes. The subsequent changes that can be detected through ultrasonography reflect the progressive phases of COVID-19. With the evolution of the disease, an increasement of B-lines can be detected. Firstly B-lines constitute the interstitial syndrome, and then, as the pneumonia advances, they represent the alveolar-interstitial syndrome and finally the white lung. Furthermore, as the inflammation progresses, in addition to B-lines, large-size consolidations, spared areas and limitation of lung sliding can be detected [52].

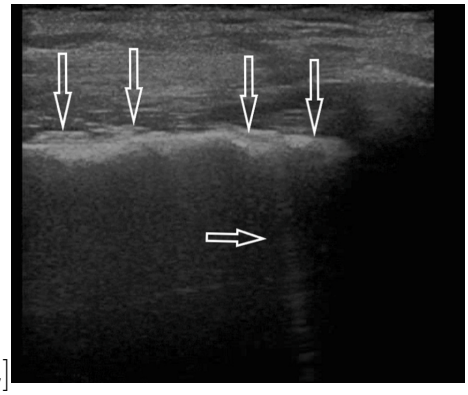
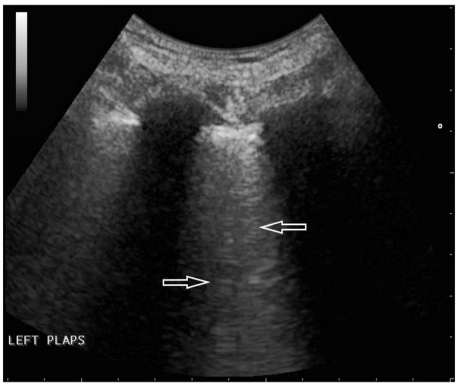
[a]  [b] 

Figure 1.19: *[a], Irregular pleural line (↓) accompanied by single B-line artifacts (→),* adapted from: Ye et al.
[b], Focally visible B-line artifacts (→, ←)., adapted from: Ye et al.

1.2.8 Risk factors

In an infectious ilness as heterogeneous as COVID-19, host's risk factors are the key to determine disease progression and severity [20].

1.2.8.1 Aging

According to actual literature, it has been proven that age is the most significant risk factor for severe COVID-19 disease and adverse health outcomes. As suggested by some studies, this assay could be linked to the age-related immune system remodeling. Effectively, immunosenescence is associated to an increased susceptibility to infection, and particularly, to respiratory infections as COVID-19 [53].

1.2.8.2 Male sex

Several studies showed a difference of mortality and morbidity between sexes. For example, H. Peckam et al. realized a meta-analysis of more than 3 milions reported cases which evidenced that males have almost three times the odds of requiring intensive treatment unit (ITU) admission than females, whereas there is no

difference in the proportion of people with confirmed COVID-19 between the two groups [54].

1.2.8.3 Comorbidities

Besides age and sex, also comorbidities are linked to the development of severe forms of COVID-19.
According to this hypothesis, several studies showed that hypertension is associated to severe form of SARS-CoV-2 and ICU care need. Also diabetes, cardiovascular disease and renal failure are linked to worst outcomes and to an increased risk of ICU admission [55].
Nonetheless, other risk factors for severe forms of COVID-19 are smoking and COPD. COPD patients demonstrated a higher expression of ACE-2 which improves the ability of SARS-CoV-2 to enter host cells and trigger the infection. COPD also contributes to the establishment of severe respiratory symptoms, including structural damage to lungs and hyper mucous production, which lead to high mortality due to blockage of air passages [56].
Other risk factors that have been proven to be associated with severe forms of COVID-19 are liver diseases, malignances and obesity. In particular, obesity is linked with the production of pro-inflammatory cytokines and adipokines, which lead to a higher immune response and hyperinflammation.
About HIV infection as a risk factor for severe COVID-19, in literature controversial hypothesis can be found, and this assay need to be investigated with further studies [16].

1.2.9 Diagnosis

About the detection of SARS-CoV-2 infection, many different types of diagnostic tests have been developed. Nevertheless, currently, the most used methods are:

- **Direct diagnosis,** based on the detection of SARS-CoV-2 genome or proteins. In particular, it could be distinguished molecular tests, which can detect viral genome and antigen tests, which can identify viral proteins.
  Among molecular tests, the main is RT-PCR, which is considered, according to the actual literature, the gold standard for the detection of SARS-CoV-2 infection [57]. Molecular tests have higher detection sensitivity compared to antigen tests. Rapid antigen test is a chromatographic immunoassay for the detection of SARS-CoV-2 nucleocapsid (N) antigen in respiratory specimens. This rapid antigen test device has two precoated lines on the result window: control (C) and test (T) lines. The control (C) region is coated with mouse monoclonal anti-chicken IgG antibody. The test (T) region is coated with mouse monoclonal anti-SARS-CoV-2 antibody against SARS-CoV-2 N antigen. Detectors for SARS-CoV-2 N antigen presented in the specimen are mouse monoclonal anti-SARS-CoV-2 antibody conjugated with color particles. The antigen–antibody color particle complex migrates via capillary force and is captured by the mouse monoclonal anti-SARS-CoV-2 antibody coated on the test (T) region. The colored test (T) line's intensity depends on the amount of SARS-CoV-2 N antigen presented in the sample [58];

- **Indirect diagnosis,** based on serological tests which can detect specific antibodies developed in the course of SARS-

CoV-2 infection. The most common antibody tests are based on enzyme-linked immunosorbent type assays (ELISA) and lateral flow type assays (LFA). Whereas nucleic acid-based tests and antigen detection tests are used for diagnostic purpose, antibody detection tests may be used for the assessment of exposure to the virus or for sero-surveillance of populations.

1.2.10 Therapy

As for the therapy of COVID-19, the first distinction to be made is between non hospitalized patients and hospitalized patients.

1.2.10.1 Non hospitalized patients

To all patients, even if they do not require hospitalization or supplemental oxygenation, symptomatic management should be offered. For patients who are at high risk of progressing to severe COVID-19, the guidelines suggest the use in order of preference of:

- Paxlovid (Ritonavir-boosted nirmatrelvir);
- Remdesivir.

The guidelines recommend against the use of dexamethasone or other systemic corticosteroids in the absence of another indication.

1.2.10.2 Hospitalized patients

About hospitalized patients, the main differentiation in pharmacological therapy is made between patients who do not require supplemental oxygen and patients who require supplemental oxygen.

1.2.10.2.1 Hospitalized patients who do not require oxygen

About hospitalized patients who do not require oxygen there is no sufficient evidence in favor or against the use of remdesivir. Nevertheless, for patients who are at high risk of disease progression, remdesivir could be given. Regarding the recommendation for anticoagulation therapy, in patients without the evidence of VTE the guidelines suggest the use of a prophylactic dose of heparin.

1.2.10.2.2 Hospitalized patients who require oxygen

Regarding the hospitalized patients who require oxygen, the various interfaces for oxygen therapy differs according to the severity of the clinical presentation of patients.

Nasal cannula

For patients presenting mild breathlessness and a SpO2 level between 94% and 97%, a simple face mask or a nasal cannula can be used for oxygen delivery.

Venturi mask

In patients maintaining a SpO2<94% or with chronic obstructive pulmonary disease (COPD) or with a respiratory rate >30/min or persistent dyspnea, oxygen is administered through a 40% Venturi mask. Reassessment is to be done after 10 minutes and, if the patient is stable, again after 6 hours. Whether SpO2 do not improve after 6 hours on a Venturi mask, the use of non-invasive ventilation (NIV) has to be taken into account.

High Flow Nasal Oxygen (HFNO)

As regards subjects where there is no improvement in dyspnea and/or SpO2<92%, even though standard oxygen therapy via face mask is administered, the use of HFNO is to be considered. The oxygen flow rate in HFNO therapy is approximately 30–40 L/min, and it needs to be adjusted according to the clinical response of the patient. Patients who do not improve after an hour with flow>50 L/min and FiO2>70% are recommended to be switched over to NIV.

Non Invasive Ventilation (NIV)

NIV by CPAP plays a primary role in the management of the respiratory failure caused by COVID-19. NIV is generally administered via oro-nasal mask or full-face mask; however, with the aim of reducing the aerosolization NIV might be also dispensed through a helmet. CPAP is started with 8–10 cmH2O and FiO2 60%, and successively these values are adjusted according to patient's compliance. In cases of hypercapnia, hemodynamic instability, multiorgan failure, abnormal mental status or worsening of oxygen saturation below 90%, invasive ventilation via endotracheal intubation has to be considered promptly [59].

Pharmacolgical therapy in hospitalized patients

- In hospitalized patients where the request for oxygen is minimal, the use of remdesivir is suggested by the guidelines. If there is a higher request for oxygen, dexamethasone alone, or combined with remdesivir is recommended. For patient on dexamethasone with rapidly increasing oxygen needs and systemic inflammation, the addition of a second immunomodulatory drug, like baricitinib (JAK inhibitor) or tocilizumab (anti-IL6R) is recommended.

- In hospitalized patients who require oxygen through HFNO or NIV, the use of dexamethasone, or dexamethasone plus remdesivir is recommended. For patient rapidly increasing oxygen needs and systemic inflammation, the addition of baricitinib or tocilizumab to one of the options mentioned above is recommended.

- In hospitalized patient who need mechanical ventilation (MV),

the use of dexamethasone is recommended. For patients who are within 24 hours of admission to the ICU, the addition of tocilizumab should be done. If tocilizumab is not available, sarilumab can be used.

Regarding the recommendation for anticoagulation therapy, in patients without the evidence of VTE the guidelines suggest the use of a prophylactic dose of LMWH (low molecular weight heparin).

It should be remembered that thanks to worldwide researches, pharmacological therapy recommended for COVID-19 treatment evolved in the course of the epidemic. Regarding this, during the first wave of SARS-CoV-2 pandemic, hydroxychloroquine was administered off-label to several patients. Although, recent studies evidenced that hydroxychloroquine does not improve the clinical outcome in COVID-19 disease, reason behind, up to date, this pharmaceutical in not any longer part of first-line treatment.

1.3 Predictors of severity and progression of COVID-19 disease

As the result of SARS-CoV-2 epidemic, an overcrowding of health care facilities and limitations of medical resources have established. As a consequence of this, in order to improve the management of patients, predictors of severity and progression of COVID-19 disease urgently need to be found. Therefore, several studies have been carried out, with the aim of stratifying

patients on the basis of death risk and odds of severe illness [60]. According to literature, COVID-19's lung damages and severity are primarily attributable to a hyperinflammation status, known as "cytokine storm". As a consequence of this, circulating hematological biomarkers could be useful in making diagnosis and predicting prognosis of COVID-19, since they can provide information about inflammation and the immune status of the host. Up to date, even though a large number of biomarkers have shown an association with COVID-19 disease severity, only some of these can predict with optimal sensitivity and specificity the outcome of patients. Among these, the most valuable are WBC, in particular neutrophils, lymphocytes, monocytes, eosinophils and also their derivative variables: NL ratio (Neutrophils to Lymphocytes ratio) and LM ratio (Lymphocytes to Monocytes ratio). Furthermore, also inflammation markers as CRP, D-dimer, ferritin, and IL-6 are actually employed as COVID-19 severity disease predictors.

1.3.1 C-reactive protein (CRP)

CRP is an acute phase protein, synthetized by the liver in response to inflammation status. Recent studies have shown that CRP blood levels are positively correlated with the severity of COVID-19 and with lung lesions [61]. Furthermore, in literature, it has been demonstrated that host factors such as gender, age, or physical condition have no impact on the CRP concentration level. As a result of this, CRP can be used as an independent biomarker for COVID-19 severity and mortality [62].

1.3.2 D-dimer

D-dimer is a fibrin degradation product, which is commonly used as an indirect marker of thrombosis. Keeping this in mind, D-dimer can be useful in the management of COVID-19, since it is currently a common knowledge that this disease is characterized by a hypercoagulable state, associated with high incidence of thromboembolic events. Indeed, according to literature, the elevation of D-dimer in severe COVID-19 patients is mainly the consequence of pulmonary thrombosis, followed by hyperfibrinolysis [63]. As for the role of D-dimer as a predictor, a recent study revealed that high level of D-dimer, both at admission and at peak, can be used as a prognostic factor for determining the risk of intubation and mortality in COVID-19 patients [64].

1.3.3 Ferritin

Ferritin represents the main intracellular storage of iron and, similarly to CRP, it is an acute phase protein whose level rises during inflammation status and immune response [65]. For this reason several studies tried to demonstrate if ferritin could be used as a predictor of COVID-19 severity. As a result of this researches, it has been proven that elevated levels of this biomarker are associated with ICU admission, intubation and higher risk of mortality [66].

1.3.4 IL-6

IL-6 is one of the main inflammatory cytokines involved in immunitary response. Therefore, a large number of studies have tried to find out the possibility of using IL-6 as a prognostic biomarker

in COVID-19, taking into account the primary role that inflammation plays in the pathogenesis of this disease. As a result of this analysis, the validity of IL-6 as a predictor of severity has been confirmed, since high serum levels of this cytokine are associated with lung injuries and prolonged mechanical ventilation [67]. Furthermore, this hypothesis is strengthened by the fact that the blockage of IL-6 receptor, by administering Tocilizumab to patients, can reduce severity of COVID-19 disease [68].

1.3.5 Neutrophils

Neutrophils are mediators of innate immune responses, which play a fundamental role in the clearance of microorganisms. On the other hand, while solving their functions, neutrophils can originate collateral tissue damages attributable to the release of cytotoxic agents and proinflammatory cytokines [69]. In COVID-19 pneumonia the hyperinflammation status is usually accompanied by an increase of neutrophils, which contribute to the lung lesions typical of this disease. Keeping this in mind, recent studies have demonstrated that the rise in neutrophil absolute count correlates positively with the severity of illness [70].

1.3.6 Lymphocytes

Lymphocytes are the main protagonists of cellular adaptive immunity. As regard lymphocyte absolute count, worldwide studies carried out that lymphopenia is a common finding in COVID-19 disease [71]. The pathophysiology of lymphopenia in COVID-19 is probably multifactorial, including:

- Direct damage of lymphocytes by virus, allowed by the ex-

pression of ACE2 on their surface;

- Invasion and destruction by SARS-CoV-2 of lymphatic organs such as thymus and spleen;
- Lymphocytes apoptosis due to excess of proinflammatory cytokine;
- Inhibition of lymphocytes by molecules such as lactic acid, resulting from metabolic disorders [72].

Due to the high prevalence of lymphopenia in COVID-19 patients, several studies have tried to find out if this feature could be a prognostic factor in the progression of disease, and the results evidenced that lymphopenia is a predictor factor of severity and poor outcomes of COVID-19 [73].

1.3.7 Eosinophils

Eosinophils are circulating and tissue-resident leukocytes that have potent proinflammatory effects, as a consequence of their preformed granules, which contains several cytotoxic proteins, first of all the Major Basic Protein. Furthermore, recent studies have carried out that eosinophils are not merely involved in inflammation status, but play an important role in immunoregulation and antiviral response [74]. In several patients with COVID-19, a decrease of eosinophil absolute count in peripherical blood can be detected. This fact is due to the accumulation of these leukocytes in the tissues (in the specific case of COVID-19, lung tissue) where they are enrolled to fight the infection. Other reasons that could explain the eosinopenia associated with COVID-19 are:

- Inhibition of eosinophils life cycle by proinflammatory cytokines;
- Apoptosis of the eosinophils induced by the release of type 1 IFN in response to SARS-CoV-2 infection;
- Depletion of eosinophils owing to the clearance of the virus [75].

As regard the eosinophil absolute count, several studies demonstrated that eosinopenia at admission is a predictor factor of severity and mortality in COVID-19 disease. On the other hand, some researches carried out a peculiar characteristic in the eosinophils trend during hospitalization, in the sense that patients with lower level of these leukocytes tend to develop higher degree of eosinophilia [76].

1.3.8 Neutrophils to Lymphocytes ratio (NLR) and Lymphocytes to Monocytes ratio (LMR)

Over the years, various studies have considered NL ratio and LM ratio as reliable prognostic markers in various clinical manifestations. These parameters are established inflammation indexes that reflect systemic inflammatory response. NL ratio and LM ratio can be calculated through a simple blood sample, which is easily accessible for almost any laboratory [77]. An increase of NL ratio has been identified in patients with severe disease, compared with subjects affected by mild illness. As for the role played by WBC, it must be remembered that neutrophils are mainly involved in the innate immune response and lymphocytes are part of adaptative immunity. Hence, an alteration of NL ratio is the reflection of an imbalance in the inflammatory response, which

can be a predictor of disease severity. Keeping this in mind, several studies underlined that an increased NL ratio in COVID-19 patients is a strong predictor for severe disease and ICU admission [78]. Regarding LM ratio, in literature there are less notions about a possible association between this index and COVID-19 severity. However, Yang et al. find out that LM ratio is significantly higher in patients with serious COVID-19 disease [79].

AIM OF THE STUDY

This is a retrospective and monocentric study which aims to evaluate the significance of hematological values in patients hospitalized for COVID-19 pneumonia in our hospital.
The first goal is to investigate a possible association between these serum values and the intensity of care that patients needed during hospitalization.
The second goal is to explore the relationship between inflammatory markers (such as NLR, CRP, D-Dimer, and eosinophil count) and COVID-19 sequelae (functional and radiological) after discharge.

MATERIALS AND METHODS

3.1 Study design and population

In this study, 327 well-characterized patients with SARS-CoV-2, referred to the University Hospital of Padua (Division of Infectious and Tropical Diseases, Respiratory Diseases Unit, and Intensive Care Unit), are retrospectively enrolled. Data are collected during the first and second pandemic waves, from February 2020 to September 2021. This study is performed following the declaration of Helsinki and is approved by the ethics committee of the University Hospital of Padua (n°46430/03082020). Clinical, radiological and demographic data are obtained at the hospital admission and at the first follow-up visit, which is conducted after 3 months from hospital discharge.

3.2 Level of medical care definition

High-intensity medical care is defined as the need for a high-flow nasal cannula (HFNC) or invasive/non-invasive ventilation (IVM/NIV); conversely, low-intensity medical care is considered when patients need only low-flow oxygen supplementation (via nasal cannula or face mask). Based on this definition, the study population was categorized into two groups, low intensity care group (n=214) and high intensity care group (n=113). For the

whole population were collected at admission:

- Clinical data (age, sex, body mass index and disease duration);
- Symptoms (fever, asthenia, dyspnea, ageusia, anosmia, musclular dizziness, alopecia, cough, gastrointestinal manifestations);
- Comorbidities (cardiovascular disease, pneumological disease, immunological disease, metabolic disease and oncological disease);
- Gas exchange values (FiO2, PaO2 and P/F);
- Blood test (Hemoglobin, White Blood Cells count, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Δ Eosinophils, Lymphocytes to Monocytes ratio, Neutrophils to Lymphocytes ratio, C-reactive protein, D-dimer and Ferritin);
- Smoking history and pack-years;
- CT scan.

Some other blood biomarkers were also calculated:

1. Lymphocytes to monocytes ratio (LMR) is obtained by dividing the absolute value of lymphocytes by the absolute value of monocytes;
2. Neutrophils to lymphocytes ratio (NLR) is obtained by dividing the absolute value of neutrophils by the absolute value of lymphocytes.

A second blood test in order to calculate the biomarkers mentioned above was obtained at the time of discharge. Hospital treatment (hydroxychloroquine, azithromycin, ceftriaxone, other antibiotics, lopinavir/ritonavir, remdesivir, tocilizumab, steroids, heparin, convalescent plasma) during hospitalization is finally reported.

Differences in eosinophils between discharge and on admission were calculated and presented as Δ eosinophils (Δ eosinophils = eosinophils at discharge minus eosinophils on admission).

At the first follow-up visit post-COVID-19, the following data are collected:

- Treatment at discharge, in particular corticosteroids;
- Respiratory functional parameters;
- Symptoms;
- CT score;
- Chest-lung ultrasound score.

3.3 Radiological evaluation

3.3.1 CT scan

Based on the radiological resolution of the lung changes, at the first follow-up visit (3 months) patients are divided into recovery group (n=168) and not-recovery group (n=159). Moreover, for a subgroup of patients (n=113) two expert thoracic radiologists blinded to clinical data, scored the images independently with

a composite semi-quantitative scale. With this method ground-glass opacities, consolidations and reticulation are analysed. For each lung lobe, the extent of ground-glass (GGO), consolidations (CONS) and reticulation/Interstitial Score (IS) are assessed using a scale from 0 to 100. Only 54 patients presented a CT scan at the time of admission and are evaluated with the same semi-quantitative score.

3.3.2 Lung ultrasound score (LUS score)

The LUS score is calculated across 12 chest zones (six on each hemithorax) using a scale from 0 (normal pattern, A-lines or non-significant B-lines), 1 (significant B-lines ≥ 3 per rib space), 2 (coalescent B-lines with or without small consolidations) to 3 (consolidation). A final "LUS global score" has been calculated for each patient, with a potential maximum score of 36 [80].

3.3.3 Statistical analysis

Descriptive statistics are used to summarise the characteristics of patients. Categorical variables are described as absolute (n) and relative values (%), whereas continuous variables as median and range (min-max). Fisher's exact test is used for categorical variables, instead Mann Whitney U test is used for quantitative variables. Wilcoxon signed-rank test was used to compare eosinophils values between admission and discharge. To assess the risk of not-REC at the first follow-up visit, univariate and multivariate regression analysis are performed. Continuous variables are dichotomized based on the median value for univariate and multivariate regression. Correlation coefficients between data are calculated using the non-parametric Spearman's rank method. All data are analysed using SPSS software version 25.0

(New York, NY, US: IBM Corp. USA) and GraphPad Prism V8 (GraphPad Software, La Jolla, CA, USA).

P-values < 0.05 were considered statistically significant.

RESULTS

4.1 Clinical characteristics of the study population

Considering the whole population, patients are more frequently males (64%) and no smokers (61%), with a median BMI of 27 (16 – 57). Moreover, the median age at SARS-CoV-2 diagnosis is 62 years (22 – 88). Other demographic characteristics of 327 patients are summarized in Table I.

We also divided patients based on the intensity of medical care: 214 patients required low-intensity medical care (LIMC) and 113 subjects needed high-intensity medical care (HIMC). These two groups don't differ in sex (64% vs 65%; p=0.92), smoking history (35% vs 45%; p=0.09) and number of pack-years (0 vs 0; p=0.27); however, patients in the HIMC group are older in comparison to LIMC patients (65 vs 60 years; p=0.01).

Regarding comorbidities, patients with HIMC present more frequently cardiological concomitant conditions (62% vs 43%; p=0.001) and metabolic disease (58% vs 40%; p=0.001). Moreover, they have reported more frequently respiratory symptoms at admission, such as dyspnea (64% VS 39%; p=0.0003), but lower anosmia/ageusia (19% vs 33%; p=0.009).

HIMC group patients had a significantly greater deterioration

	Overall (327)	LIMC (214)	HIMC (113)	p-value
Age – years	62 (22 – 88)	60 (22 – 87)	65 (25 – 88)	**0.01**
Sex – male, n° (%)	210 (64%)	137 (64%)	73 (65 %)	0.92
BMI (Kg/m2)	27 (16 – 57)	26 (16 – 57)	27 (19 – 48)	0.19
Pack-Years	0 (0 – 90)	0 (0 – 66)	0 (0 – 90)	0.27
Smoking History – n° (%)	126 (39%)	75 (35%)	51 (45%)	0.09
Comorbidities				
• ***Cardiological – n° (%)***	162 (50%)	92 (43%)	70 (62%)	**0.001**
• ***Pneumological – n° (%)***	51 (16%)	37 (21%)	14 (12%)	0.24
• ***Immunologic – n° (%)***	45 (14%)	29 (16%)	16 (16%)	0.88
• ***Metabolic – n° (%)***	151 (46%)	85 (40%)	66 (58%)	**0.001**
• ***Oncologic – n° (%)***	54 (17%)	31 (14%)	23 (20%)	0.17
Symptoms				
• ***Fever – n° (%)***	303 (93%)	194 (91%)	109 (96%)	0.06
• ***Asthenia – n° (%)***	117 (36%)	81 (38%)	36 (32%)	0.28
• ***Dyspnea – n° (%)***	156 (48%)	84 (39%)	72 (64%)	**0.0003**
• ***Cough – n° (%)***	184 (56%)	120 (56%)	64 (57%)	0.92
• ***Anosmia/Ageusia – n° (%)***	93 (28%)	71 (33%)	22 (19%)	**0.009**
• ***Muscular Dizziness – n° (%)***	60 (18%)	39 (18%)	21 (19%)	0.94
• ***GI Symptoms– n° (%)***	71 (22%)	44 (21%)	27 (24%)	0.49
FiO2 At Admission	21 (21 – 100)	21 (21 – 88)	29 (21 – 100)	**<0.0001**
PaO2 At Admission	68 (21 – 150)	71 (49 – 145)	61 (21 – 150)	**<0.0001**
P/F	283 (40 – 542)	309 (121 – 542)	224 (40 – 461)	**<0.0001**
CT Scan at admission				
- Alveolar score	7 (0 – 62)	5 (0 – 38)	18 (0 – 62)	**0.01**
- Consolidation score	1 (0 – 26)	0.8 (0 – 10)	4 (0 – 26)	**0.001**
- Interstitial score	1.6 (0 – 29)	0.8 (0 – 29)	10 (0 – 23)	**0.001**
Hospitalization (days)	11 (2 – 67)	8 (2 – 49)	18 (3 – 67)	**<0.0001**

Table I: **Demographics and clinical characteristics of the overall population, of patients of the low-intensity medical care group and of patients of the high-intensity medical care group.**
(BMI: body mass index, GI: gastrointestinal, CT scan: computer tomography. Values are expressed as numbers and (%) or median and range, as appropriate. To compare demographics between HIMC and LIMC, the chi-square test and Fisher's t-test for categorical variables and Mann–Whitney t-test for continuous variables were used)

of respiratory gas exchange, with a higher FiO2 request, (29 vs 21; p<0.0001) and a worse PaO2 on room air (61 vs 71 mmHg; p<0.0001) at hospital admission.

Considering radiological evaluation during hospitalization, subjects with LIMC present a lower rate of alveolar score (5 vs 18; p=0.01), consolidation score (0.8 vs 4; p=0.001) and interstitial score (0.8 vs 10; p=0.001) in comparison to subjects of the HIMC group.

4.2 Blood tests in the study population

Inflammatory indexes and blood cell count were considered at admission. Therefore, patients of the HIMC group have higher white blood cell count (6.9 vs 5.46; p<0.0001), neutrophils (5.68 vs 3.97; p<0.0001), NLR (7.28 vs 4.05; p<0.0001), CRP (98 vs 43; p<0.0001) and ferritin (806 vs 529; p<0.0001).

	Overall (327)	**LIMC (214)**	**HIMC (113)**	**p-value**
Blood test at admission				
• ***Hemoglobin (g/L)***	133 (75 – 177)	136 (88 – 177)	130 (75 – 168)	**0.008**
• ***WBC (x10^9/L)***	6 (1.42 – 25.6)	5.46 (1.42 – 25.6)	6.9 (1.63 – 19.15)	**<0.0001**
• ***Neutrophils (x10^9/L)***	4.25 (1.05 – 22.7)	3.97 (1.05 – 22.7)	5.68 (1.08 -19)	**<0.0001**
• ***Lymphocytes (x10^9/L)***	0.92 (0.09 – 4.18)	0.98 (0.19 – 4.18)	0.73 (0.09 – 3.69)	**<0.0001**
• ***Monocytes (x10^9/L)***	0.43 (0.02 – 1.83)	0.46 (0.02 – 1.83)	0.38 (0.02 – 1.71)	**0.02**
• ***Eosinophils (x10^9/L)***	0 (0 – 0.24)	0 (0 – 0.24)	0 (0 – 0.14)	**<0.0001**
• ***LM Ratio***	2.16 (0.37 – 17)	2.23 (0.51 – 11.8)	1.97 (0.37 – 17)	0.14
• ***NL Ratio***	4.64 (0.73 – 93.45)	4.05 (0.73 – 41)	7.28 (1.27 – 93.45)	**<0.0001**
• ***CRP (mg/dl)***	59.5 (2.9 - 350)	43 (2.9 – 270)	98 (3.3 – 350)	**<0.0001**
• ***D-Dimer (mcg/ml)***	169 (150 - 26732)	164 (150 – 26732)	187 (150 – 24774)	0.07
• ***Ferritin (ng/ml)***	589 (8.3 – 12057)	529 (8.3-4723)	806 (17-12057)	**<0.0001**
Blood test at discharge				
• ***Hemoglobin (g/L)***	127 (78 – 175)	130 (88 – 173)	118 (78 – 175)	**<0.0001**
• ***WBC (x10^9/L)***	7.41 (1.77 – 17.5)	7.3 (1.77 – 17.5)	7.46 (2.71 – 15)	0.48
• ***Neutrophils (x10^9/L)***	4.58 (0.21 – 14.84)	4.6 (0.21 – 14.84	4.43 (0.96 – 12.8)	0.81
• ***Lymphocytes (x10^9/L)***	1.71 (0.18 – 8.71)	1.62 (0.18 – 4.6)	1.84 (0.4 – 8.71)	**0.04**
• ***Monocytes (x10^9/L)***	0.66 (0.05 – 1.79)	0.66 (0.17 – 1.79)	0.66 (0.05 – 1.35)	0.78
• ***Eosinophils (x10^9/L)***	0.06 (0 – 0.72)	0.05 (0 – 0.34)	0.1 (0 – 0.72)	**<0.0001**
• ***LM Ratio***	2.63 (0.62 – 17.4)	2.57 (0.62 – 9)	2.89 (0.67 – 17.4)	**0.02**
• ***NL Ratio***	2.45 (0.06 – 35.2)	2.56 (0.06 – 35.2)	2.14 (0.38 – 15)	0.10
• ***CRP (mg/dl)***	6.05 (2.9 – 150)	6.7 (2.9 – 150)	4.8 (2.9 – 110)	**0.003**
• ***D-Dimer (mcg/ml)***	191 (150 – 6248)	166 (150 – 5590)	213 (150 – 6248)	**0.006**
• ***Ferritin (ng/ml)***	723 (15 – 2067)	612 (15 – 1797)	822 (74 – 2067)	**0.04**
Δ ***eosinophils***	0.05 (-0.15 – 0.72)	0.04 (-0.15 – 0.3)	0.1 (-0.03 – 0.72)	**<0.0001**

Table II: **hematological values collected at admission and discharge in whole patient population (327) and in dividing patients in LIMC (214) and HIMC (113).**
(WBC: white blood cells, LM: lymphocytes-to-monocytes ratio, NL: neutrophils-to-lymphocytes ratio. Values are expressed as numbers and (%) or median and range, as appropriate. To compare demographics between HIMC and LIMC, the chi-square test and Fisher's t-test (n < 5 >) for categorical variables and Mann–Whitney t-test for continuous variables were used.)

Conversely, patients of the LIMC group show a higher lymphocyte count (0.98 vs 0.73; p<0.0001), eosinophil count (0 vs 0; p<0.0001), and monocyte count (0.46 vs 0.38; p=0.02); whereas no differences were observed for D-dimer (164 vs 187; p=0.07) and LMR (2.23 vs 1.97; p=0.14).

A second blood test was collected at discharge from the hospital ward and no differences are detected for WBC count (7.3 vs 7.46; p=0.48), neutrophils (4.6 vs 4.43; p=0.18) and monocytes (0.66 vs 0.66; p=0.78). Differently from the first blood sample, both lymphocytes (1.84 vs 1.62; p=0.04) and eosinophils (0.1 vs 0.05; p<0.0001) are higher in the HIMC group. In comparison to HIMC, ferritin (612 vs 822; p=0.04) and D-dimer (166 vs 213; p=0.006) are lower in the LIMC group, but the latter present a higher CRP level (6.7 vs 4.8; p=0.003).

We further analysed eosinophils' trend from admission to discharge: the low eosinophil count at admission reached normal values in both groups, however in the HIMC group eosinophils reached significantly higher levels as compared with LIMC patients (0.1 vs 0.04; p<0.0001). Figure 1 reports eosinophils' change from admission to discharge in the two groups (HIMC and LIMC).

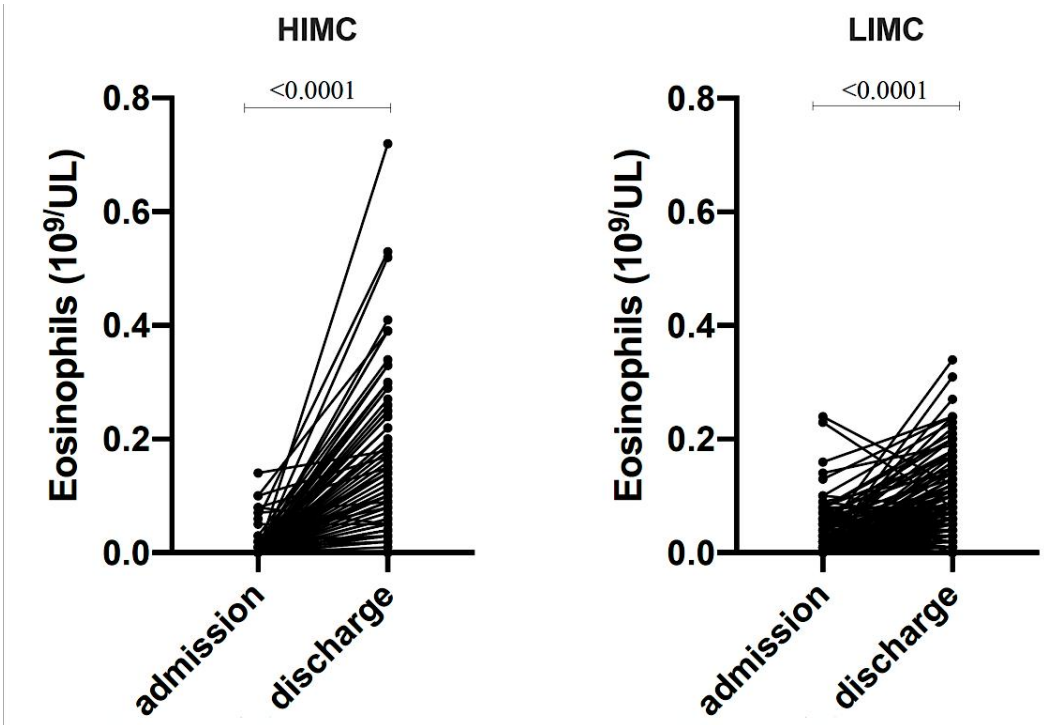


Figure 4.1: Eosinophils trends from admission to hospital discharge in HIMC and LIMC groups. Wilcoxon signed-rank test was used to compare eosinophils values between admission and discharge in HIMC patients ($p<0.0001$) and LIMC patients ($p<0.0001$).

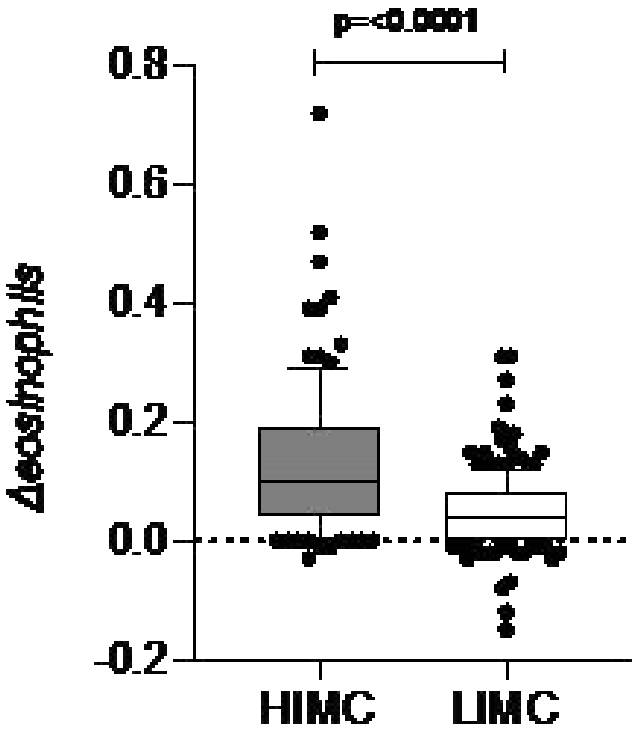


Figure 4.2: Δ eosinophils of the two groups categorized according to the intensity of care during hospitalization. Horizontal bars represent median values; bottom and top of each box plot 25th and 75th ($p<0.0001$).

4.3 Treatment

During hospitalization, treatment changed accordingly to the severity of the disease. In particular, patients with HIMC received more frequently Tocilizumab (12% vs 2%; p=0.0002), corticosteroids (83% vs 68%; p=0.003) and heparin (92% vs 82% p=0.03). Furthermore, in both groups similar percentage of patients received azithromycin (56% vs 63%; p=0.20) and ceftriaxone (49% vs 37%; p=0.49); in addiction patients of HIMC group received more frequently antibiotics (64% vs 18%; p<0.0001).

	LIMC (214)	HIMC (113)	p-value
Therapy During Hospitalization			
• ***Hydroxychloroquine/Chloroquine***	72 (34%)	45 (40%)	0.17
• ***Azithromycin***	135 (63%)	63 (56%)	0.20
• ***Ceftriaxone***	80 (37%)	55 (49%)	0.49
• ***Other Antibiotics***	39 (18%)	72 (64%)	**<0.0001**
• ***Lopinavir/Ritonavir***	35 (16%)	25 (22%)	0.20
• ***Remdesivir***	75 (35%)	39 (35%)	0.92
• ***Other Antivirals***	3 (1%)	3 (3%)	0.42
• ***Tocilizumab***	4 (2%)	13 (12%)	**0.0002**
• ***Corticosteroids***	145 (68%)	94 (83%)	**0.003**
• ***Heparin***	176 (82%)	103 (92%)	**0.03**
• ***Convalescent Plasma***	46 (21%)	27 (24%)	0.62
Treatment After Discharge			
• ***Corticosteroids***	121 (57%)	75 (66%)	0.08

Table III: **Treatment during hospitalization of the overall population evaluated at the post-COVID clinic, and of the two groups of patients categorized according to the presence of radiological recovery during the follow-up period.**

4.4 First follow-up

Patients were evaluated at the post-COVID clinic after 3 months from discharge.

In the overall population, the median FVC in liters is 3.37 (1.46 – 7.96) and the median FEV1 in liters is 2.83 (0.84 – 6.11). In the whole population, the median FVC is 92% predicted (45 – 136) and the median FEV1 is 95% predicted (31 – 137). Despite lung function being normal in both groups, we find a lower FEV1 (%predicted) (92% vs 96%; p=0.05) and FVC (%predicted) (87% vs 93%; p=0.001) in HIMC group.

In the HIMC group compared with LIMC, we observe a higher percentage of patients with persistent lung damage at first follow-up visit (not-REC group) (64% vs 41%; p=0.0007) and with higher radiological involvement in the first CT scan after discharge (alveolar score: 3.2 vs 0.6; p=0.005; interstitial score: 0 vs 0; p=0.01).

During the follow-up visit both groups of patients show the same symptoms, except for muscular dizziness which is more prevalent in HIMC patients (21% vs 12%; p=0.02).
During follow-up, we also performed lung ultrasonography (LUS), where we found that the ultrasound score was increased in HIMC patients, in comparison with LIMC subjects (2 vs 1; p<0.0001).

	Overall (327)	**LIMC (214)**	**HIMC (113)**	**p-value**
First follow-up visit after discharge				
• ***FEV1 (L)***	2.83 (0.84 – 6.11)	2.8 (0.84 – 6.11)	2.86 (1.09 – 4.25)	0.26
• ***FEV1 (%pred)***	95 (31 – 137)	96 (31 – 137)	92 (53 – 130)	**0.05**
• ***FVC (L)***	3.37 (1.46 – 7.96)	3.48 (1.46 – 7.96)	3.23 (1.57 – 5.09)	0.06
• ***FVC (%pred)***	92 (45 – 136)	93 (45 – 136)	87 (58 – 119)	**0.001**
• ***LUS score***	1 (0 – 15)	1 (0 – 9)	2 (0 – 15)	**<0.0001**
Not-Rec CT scan (yes)	159 (49%)	87 (41%)	72 (64%)	**0.0007**
CT scan at first follow-up				
• ***Alveolar score***	1.4 (0 – 68)	0.6 (0 – 26)	3.2 (0 – 68)	**0.005**
• ***Consolidation score***	0 (0 – 10)	0 (0 – 7)	0 (0 – 10)	0.80
• ***Interstitial score***	0 (0 – 18)	0 (0 – 16)	0 (0 – 18)	**0.01**
Symptoms at first follow-up visit				
• ***Asthenia***	144 (44%)	94 (44%)	50 (44%)	0.96
• ***Dyspnea***	100 (31%)	62 (29%)	38 (34%)	0.38
• ***Cough***	29 (9%)	18 (8%)	11 (10%)	0.69
• ***Anosmia/Ageusia***	22 (7%)	17 (8%)	5 (4%)	0.23
• ***Muscular Dizziness***	51 (16%)	26 (12%)	25 (21%)	**0.02**
• ***GI Symptoms***	4 (1%)	2 (1%)	2 (2%)	0.51

Table IV: **clinical and functional characteristics at first follow-up (3 months) of the overall population (327) and patients divided into LIMC (214) and HIMC (113).**
FVC: forced vital capacity, FEV1: flow expiratory volume in the first second, GI: gastrointestinal. Values are expressed as numbers and (%) or median and range, as appropriate. To compare demographics between HIMC and LIMC, the chi-square test and Fisher's t-test (n < 5) for categorical variables and Mann–Whitney t-test for continuous variables were used.

4.5 Prognostic factors for radiological sequelae at follow-up

To detect predictors for not-REC at the first CT scan, logistic regression was performed. In the univariate analysis age $\geq$ 62 years (p=0.002), a high degree of medical care (p=0.0001), NL ratio at admission $\geq$ 4.64 (p=0.02), neutrophils at admission $\geq$ 4.25 x$10^9/L$ (p=0.002), CRP at admission $\geq$ 59.5 (mg/dl) (p=0.007),

ferritin at admission $\geq$ 589 (ng/ml) (p=0.04), Δ eosinophils $\geq$ 0.05 (p=0.002) and oncological diseases (p=0.04) are associated with persistent radiological abnormalities at follow-up.
In multivariate analysis, age $\geq$ 62 years (p=0.03) and Δ eosinophils $\geq$ 0.05 (p=0.03) are two independent predictor factors of radiological lung sequelae in the whole patients population.

	Univariate		**Multivariate**	
	HR(0.95CI)	P	HR(0.95CI)	P
Age $\geq$ 62 Years	2.04 (1.3 – 3.2)	**0.002**	1.75 (1.05 – 2.94)	**0.03**
Sex – Male	1.37 (0.87 – 2.16)	0.17	-	-
BMI $\geq$ 27 (Kg/m^2)	0.95 (0.59 – 1.50)	0.82	-	-
Pack - Years $\geq$ 0	1.04 (0.66 – 1.65)	0.85	-	-
Severity - HIMC	2.56 (1.60 – 4.10)	**0.0001**	1.53 (0.86 – 2.72)	0.15
Pre-Admission Hematological Values				
• ***LM Ratio $\geq$ 2.16***	1.23 (0.79 – 1.91)	0.35	-	-
• ***NL Ratio $\geq$ 4.64***	1.66 (1.07 – 2.58)	**0.02**	0.87 (0.47 – 1.62)	0.66
• ***Neutrophils $\geq$ 4.25 (x10^9/L)***	2.03 (1.31-3.16)	**0.002**	1.40 (0.78 – 2.53)	0.26
• ***Lymphocytes $\geq$ 0.92 (x10^9/L)***	0.89 (0.58-1.39)	0.62	-	-
• ***Monocytes $\geq$ 0.43 (x10^9/L)***	0.87 (0.57-1.35)	0.55	-	-
• ***Eosinophils $\geq$ 0 (x10^9/L)***	0.78 (0.49 - 1.23)	0.28	-	-
• ***CRP $\geq$ 59.5 (mg/dl)***	1.85 (1.18 – 2.89)	**0.007**	1.04 (0.57 – 1.88)	0.89
• ***D-Dimer $\geq$ 169 (mcg/ml)***	1.21 (0.77 – 1.90)	0.42	-	-
• ***Ferritin $\geq$ 589 (ng/ml)***	1.66 (1.03 – 2.66)	**0.04**	1.50 (0.84 – 2.49)	0.18
Pre-Discharger Haematological Values				
• ***LM Ratio $\geq$ 2.63***	1.2 (0.78 – 1.86)	0.40	-	-
• ***NL Ratio $\geq$ 2.45***	0.92 (0.59 – 1.42)	0.70	-	-
• ***Neutrophils $\geq$ 4.58 (x10^9/L)***	1.36 (0.88 – 2.10)	0.17	-	-
• ***Lymphocytes $\geq$ 1.17 (x10^9/L)***	1.39 (0.90 – 2.16)	0.13	-	-
• ***Monocytes $\geq$ 0.66 (x10^9/L)***	1.54 (0.99 – 2.39)	0.051	-	-
• ***Δ Eosinophils $\geq$ 0.05***	2.03(1.30 – 3.17)	**0.002**	1.75 (1.05 – 2.9)	**0.03**
• ***CRP $\geq$ 6.00 (mg/dl)***	0.92 (0.58 – 1.46)	0.72	-	-
• ***D-Dimer $\geq$ 191 (mcg/ml)***	1.27 (0.73 – 2.2)	0.39	-	-
• ***Ferritin $\geq$ 723 (ng/ml)***	2.25 (0.68 – 7.41)	0.18	-	-
Cardiological – yes	1.29 (0.84 – 1.99)	0.25	-	-
Oncological – yes	2.44 (1.32 – 4.51)	**0.04**	1.8 (0.9 – 3.7)	0.09
Pneumological – yes	1.12 (0.61 – 2.03)	0.71	-	-
Metabolic – yes	1.03 (0.67 – 1.59)	0.89	-	-
Autoimmunity – yes	1.53 (0.81 – 2.89)	0.19	-	-

Table V: **Predictive factors of radiological sequelae at follow-up in patients hospitalized for SARS-COV-2-related pneumonia.**
(BMI: body mass index, GI: gastrointestinal, WBC: white blood cells, LM: lymphocytes-to-monocytes ratio, NL: neutrophils-to-lymphocytes ratio, CPR: C-Reactive Protein; HIMC: high-intensity medical care. Values are expressed as numbers and (%) or median and range, as appropriate)

4.6 Correlations

Based on the result from univariate and multivariate analysis, we performed multiple correlations, at first, between NLR and func-

tional parameters, and then between NLR and radiological score. The same correlation was made with Δ eosinophils.

Both NLR at admission and Δ eosinophils positively correlate with alveolar score (r=0.30, p=0.002; r=0.20, p=0.04; respectively) and interstitial score (r=0.22, p=0.02; r=0.27, p=0.003 respectively) at first CT scan after discharge (3 months). Similarly, a positive correlation has been observed between lung ultrasound score at first follow-up, NLR and Δeosinophils (r=0.13, p=0.02; r=0.14, p=0.02 respectively).

NLR at admission correlates negatively with functional parameters at the first follow-up visit (FVC %pred; r=-0.18, p=0.002; FEV1 %pred; r=-0.14, p=0.02).

Moreover, in the HIMC group, a negative correlation is present between Δ eosinophils and FVC (L) (r=-0.22; p=0.03), as reported in Figure 3.

	NLR at admission		**Δ eosinophils**	
	R	**p**	**r**	**p**
FU first follow up				
• ***FVC (Pred%)***	-0.18	**0.002**	-0.06	0.28
• ***FEV1 (Pred%)***	-0.14	**0.02**	-0.02	0.70
• ***LUS score***	0.13	**0.02**	0.14	**0.02**
CT scan first follow up				
• ***Alveolar score***	0.30	**0.002**	0.20	**0.04**
• ***Consolidation score***	0.07	0.48	0.20	0.83
• ***Interstitial score***	0.22	**0.02**	0.27	**0.003**

Table VI: **Table 6: correlation between NLR at admission, Δ eosinophils with functional and radiological values at first follow-up.**
(FVC: forced vital capacity, FEV1: flow expiratory volume in the first second, CT: computer tomography.)

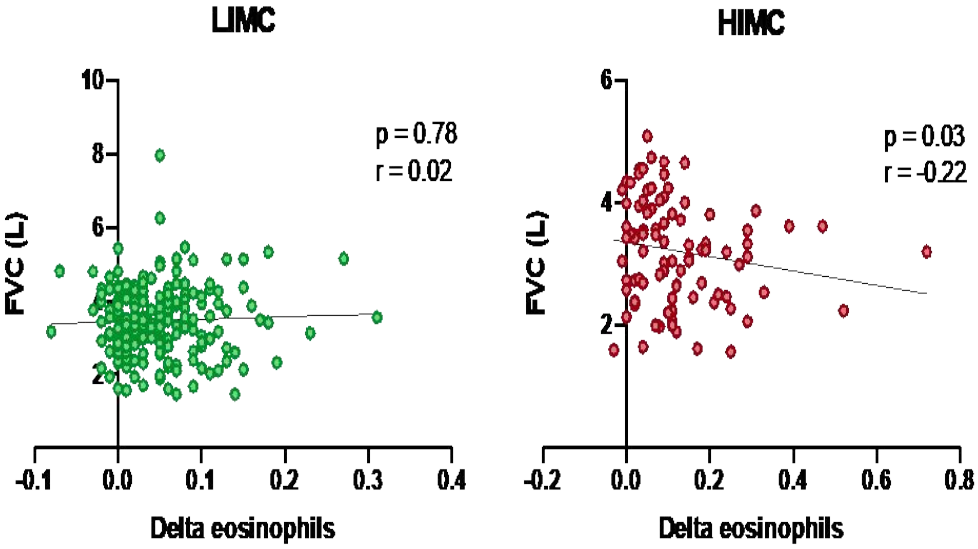


Figure 4.3: Correlation analysis between FVC (L) and eosinophils in LIMC (r=0.02; p=0.78) and HIMC (r=-0.22; p=0.03) groups.

DISCUSSION

In this retrospective study, we evaluate the role of hematological values in patients hospitalized for COVID-19 pneumonia in our hospital.

We obtained a total of 327 patients, 214 of them were classified as LIMC and 113 as HIMC.

In the LIMC group patients were younger [60 (22 – 87); 65 (25 – 88); p=0.01] and with lower cardiological (43% vs 62%; p=0.001) and metabolic disorders (40% vs 58%; p=0.001) compared with HIMC.

Moreover, the LIMC group needed lower FiO2 at admission [21 (21 – 88); 29 (21 – 100); p<0.0001] as they presented higher PaO2 [71 (49 – 145); 61 (21 – 150); p<0.0001] and P/F [309 (121 – 542); 224 (40 – 461); p<0.0001].

The two groups are similar in terms of symptoms at admission, such as fever, asthenia, and cough; on the other hand HIMC patients had a higher rate of dyspnea (64% vs 39%; p=0.0003), and a lower rate of anosmia/ageusia (19% vs 33%; p=0.009) in comparison to LIMC group.

Concerning blood values at admission, WBC count [6.9 (1.63 – 19.15); 5.46 (1.42 – 25.6); p<0.0001], neutrophils [5.68 (1.08 - 19); 3.97 (1.05 – 22.7); p<0.0001], NLR [7.28 (1.27 – 93.45); 4.05 (0.73 – 41); p<0.0001)] and CRP [98 (3.3 – 350); 43 (2.9 – 270);

p<0.0001] were higher in HIMC compared to LIMC. Contrariwise, the LIMC group had a higher level of eosinophils [0 (0 – 0.24); 0 (0 – 0.14); p<0.0001] and monocytes [0.46 (0.02 – 1.83); 0.38 (0.02 – 1.71); p=0.02] at admission.

Interestingly, several authors evaluated the role of NLR and eosinophils as prognostic biomarkers in patients with COVID-19. Yu-Qing Cai and co-workers reported that high levels of NLR, LDH, D-dimer, and CT scores were significantly correlated with COVID-19 severity [81].

Furthermore, in a study conducted by Jimeno S. et al. in 119 patients with COVID-19, authors reported a higher NLR at baseline and a higher peak of NLR in severe clinical courses. In their multivariate logistic regression analysis age, CRP at admission and peak NLR were significantly associated with a higher risk of death [82].

In addition, in non-survivors, Yan et al. reported a lower number of lymphocytes and increased neutrophils with a consequent elevation of NLR. It is well established that neutrophils are mainly involved in the innate immune response and lymphocytes are part of adaptative immunity. Hence, an alteration of the NLR is the reflection of an imbalance in the inflammatory response that occurs in COVID-19 patients and in other infectious diseases. Authors argued that high NLR at admission could represent a strong predictor for in-hospital mortality in patients with COVID-19 infection [78].

In our analysis, NLR at admission was higher in patients with HIMC [7.28 (1.27 – 93.45); 4.05 (0.73 – 41); p<0.0001] and positively correlated with the alveolar (r=0.30; p=0.002) and the interstitial score (r=0.22; p=0.02) in the first CT scan after hospitalization.

As concern blood tests at discharge, a recovery of neutrophil count and eosinophil count have been found in both groups (Table 2). However, we noticed an unexpected eosinophils rising in the HIMC group in comparison to the LIMC group (Figure 1). For that reason, we calculated the Δ eosinophils, with the same method used by Chen et al. (Δ eosinophils= eosinophils at discharge minus eosinophils on admission) [83], in the aim of finding out if this index may be used as a predictor for not-recovery at the first follow-up visit (3 months), as showed in Figure 2.

In HIMC patients, the median Δ eosinophils from admission to discharge was 0.1 (-0.03 – 0.72), whereas patients with LIMC had 0.04 (-0.15 – 0.3) ($p<0.0001$). Fraissè et al. reported in their study an unexpected eosinophilia in critically ill patients; that finding was a late-onset event in the course of ICU stay, and this could have a positive impact on survival.

However, this is difficult to interpret, because patients developing eosinophilia were exposed to a survival bias. Our results support this hypothesis. Fraissè et al. also speculated that SARS-CoV-2 was directly or indirectly responsible for eosinophilia, as a consequence of infection or recovery [84].

However, we evaluate only alive patients and not dead patients. Surprisingly, our findings suggest also an increased risk of pulmonary sequelae in terms of fibrotic residuals in patients with a higher increase of eosinophil count in comparison to a normal eosinophil rise, regardless of the level of medical care.

In our univariate and multivariate analysis, higher eosinophils rise during hospitalization and older age are two independent risk factors of pulmonary sequelae at the first follow-up CT scan [1.75 (1.05 – 2.94); p=0.03 and 1.75 (1.05 – 2.9); p=0.03].

In correlation analysis, a higher Δ eosinophils positively correlates

with interstitial (r=0.27; p=0.003) and alveolar scores (r=0.20; p=0.04) in the first CT scan after discharge and also with LUS score at the first follow-up visit (r=0.14; p=0.02). This is in line with Yang Zhen Lu et al., since they reported higher level of eosinophil count in COVID-19 patients with evidence of fibrotic change. Moreover, high total score on peak CT, eosinophil count, ESR and advancing age were related to fibrotic change on CT at the early recovery stage in patients with COVID-19 [85].

Furthermore, Toraldo and coworkers reported the same in their study conducted in 75 patients with COVID-19. Eosinophil count, IL-6 and GPT showed a significant association with the presence of radiological sequelae at month 3 [86]. Several other studies suggest the role of eosinophils in the release of pro-fibrotic cytokines. In particular, IL-5 can promote fibrosis in the lung by recruiting eosinophils that produce TGF-β1, PDGF, and IL-13 [87].

It is also known that persistent low eosinophil count might be an ominous sign of severe disease and a higher risk of death [88]. Maybe our HIMC patients have an increase in the eosinophils count and this is positive because they survive. On the other hand, an extra-rising can induce an over-protective repair, consisting in more radiological sequelae.

Of course, our study has some limitations: first of all, the retrospective and monocentric characteristics may limit the strength of the results. Second, in our cohort, data from dead patients were not detected. Third, no other blood tests were considered during the hospital stay, thus we did not evaluate the level of changes in blood values in the course of the disease. Further, larger studies are needed to overcome these limitations.

CONCLUSIONS

In conclusion, we found that NLR at admission and higher Δ eosinophils positively correlate with radiological score (interstitial and alveolar) at first CT scan after discharge (3 months). Moreover, older age and Δ eosinophils ≥ 0.05 are two independent factors of radiological sequelae in post COVID CT scan. Based on our findings, Δ eosinophils and NLR at baseline could be potential predictors of radiological sequelae in CT scan, even though further studies are needed to investigate the role of blood values in post COVID-19 sequelae.

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