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SARS-COV-2 INFECTION; PATHOPHYSIOLOGICAL MECHANISMS; THE ROLE OF SECTIONAL IMAGING IN THE EVALUATION AND MONITORING OF PATIENTS

SUMMARY OF THE DOCTORAL THESIS

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1. Introduction

The pandemic caused by the SARS-CoV-2 virus has had a major impact on the healthcare system, economy, and society, with isolation measures, quarantine, travel restrictions, hospital overcrowding, and overburdened medical staff. Successive waves of infections have significantly increased the number of patients requiring medical care, some of whom suffer serious complications, with increased mortality.

Medical imaging played an essential role during the COVID-19 pandemic, significantly contributing to the diagnosis, monitoring, and management of infected patients. Chest X-rays and computed tomography (CT) scans were widely used to identify characteristic signs of infection, such as ground-glass opacities and pulmonary consolidations. CT scans provided a detailed assessment of the extent and severity of pulmonary involvement, allowing doctors to classify the severity of the disease and decide on appropriate treatment, as well as to monitor the progression of the disease in hospitalized patients, evaluating their response to treatment and identifying complications such as pneumonia or pulmonary embolism. The onset of fibrotic changes in "long-COVID" patients also represents an important role that imaging can play in managing comorbidities that develop post-infection.

Thus, after accumulating an advanced understanding of the pathophysiological mechanisms of SARS-CoV-2 infection, the research hypothesis centered around the idea of testing the connection between imaging findings (patterns and degree of involvement) and the clinical-biological profile, focusing attention on non-pulmonary involvements, specifically cardiovascular, hepatobiliary, and thrombotic types. Although imaging investigations performed on COVID-19 patients primarily aimed to quantify pulmonary involvement, valuable information about non-pulmonary involvement can be extracted from these examinations. Considering the difficulties in accessing and mobilizing patients to imaging laboratories during the pandemic, extracting maximum information from radiological examinations is a benefit for all participants and beneficiaries of the medical act.

The research objectives started with presenting the common and less common patterns of pulmonary involvement in COVID-19 patients visible on computed tomography (CT) scans, subsequently establishing correlations between the degree and modes of pulmonary involvement and the clinical-biological data of patients at disease onset. These objectives focused on non-pulmonary involvement, specifically cardiovascular,

hepatobiliary, and thromboembolic, as well as calculating risk scores and prediction models to develop such complications.

2. Research Methodology

The initial study cohort included adult patients with a positive real-time Polymerase Chain Reaction (rt-PCR) test and one or more computed tomography (CT) imaging examinations within the first two days of hospitalization. Exclusion criteria were pediatric age, pregnant or breastfeeding women, and patients with known cardiac, renal, or hepatic impairment, as well as chronic pulmonary thromboembolism. Consequently, the final cohort consisted of 479 patients, of whom 46 (9%) underwent two or more imaging examinations during hospitalization, including one within the first two days.

Among the selected clinical and biological data were gender, age, hospitalization duration, heart and respiratory rates at admission, biochemical markers of cardiac and hepatic impairment, inflammation markers, as well as coagulation and fibrinolysis markers.

Several types of pulmonary involvement visible on imaging were defined according to the literature, with semi-quantitative grading based on severity or extent. This involved tracking the number of pulmonary segments with interstitial involvement, the number of pulmonary segments with alveolar consolidations, the extent of interstitial thickening, vascular ectasias, or fibro-atelectatic changes, as well as the presence or absence of pericardial or pleural effusions, or mediastinal lymphadenopathy.

3. GENERAL PART

3.1. Infection Mechanisms and Immune Responses in SARS-CoV-2 Infection

The most well-known pathway for SARS-CoV-2 to infect cells is mediated by the ACE2 (angiotensin-converting enzyme 2) receptor through the S protein. This protein has a high affinity for the mentioned receptor, leading to rapid infection of cells with a significant representation of this receptor. Thus, we note a high density of receptors in the myocardium, endothelium, intestines (via enterocytes), kidneys, brain, and testes.

Innate immunity plays a critical role in defending against SARS-CoV-2 (Zhang, et al., 2020) through chemokines, related receptors, and interferon-dependent pathways. In this context, pro-inflammatory macrophages, which express high levels of ACE2, have a multifaceted significance: they release pro-inflammatory chemokines, recruiting and activating T cells that produce interferon and cytokines, which in turn further activate macrophages. This creates positive feedback loops that induce heightened inflammation, triggering strong inflammatory responses in COVID-19 pathology.

Acquired immunity represents specific pathogen immunity following initial contact, ensuring long-term immune memory. B lymphocytes play a critical role in this type of immunity by producing various types of antibodies involved in the acquired immune response. IgM antibodies have a lower affinity for viral particles compared to IgG, which is also true in SARS-CoV-2 infection. Immunoglobulin maturation is an important step in acquiring immunity and resolving the ongoing inflammatory-infectious process during the disease, especially in mild and moderate forms.

3.2. Respiratory System Involvement

Patients can be asymptomatic or present with signs and symptoms suggesting an upper respiratory tract infection, disturbances in smell and taste, to severe forms of bronchopneumonia and acute respiratory distress syndrome (ARDS). ACE2 receptors are the main target of the SARS-CoV-2 virus and are well represented on the surfaces of type 2 alveolar cells, ciliated cells, and goblet cells (Jacobs, et al., 2020). Chronic obstructive pulmonary disease (COPD) is the most significant risk factor for severe forms according to (Alqahtani, et al., 2020). Autopsy examinations (Zhang, et al., 2020) have observed diffuse alveolar damage, exudative and interstitial inflammation in ARDS cases. The pulmonary interstitium was characterized by blood vessels with edematous walls, monocytic, lymphocytic infiltration, and disseminated macrophages. Additionally, focal intra-alveolar

hemorrhage and intra-alveolar fibrinous exudates were identified in patches, along with the formation of hyaline membranes and partial exfoliations of the bronchial epithelium.

3.3. Cardiovascular System Involvement

Cardiovascular complications associated with SARS-CoV-2 viral pneumonia vary in incidence depending on the studied population patterns, ranging from 40% (Chen, et al., 2020) to 2-4% (Guan, et al., 2020). An important aspect is the population with pre-existing cardiovascular conditions, especially in light of the therapeutic regimens used and how these may or may not influence the severity of the viral infection in COVID-19. For example, pharmacological inhibitors of the renin-angiotensin system (RAS) increase ACE2 levels, facilitating the ease and severity of infection (Hanff, et al., 2020). Myocarditis is one of the most common cardiovascular complications in COVID-19, being 13 times more frequent in patients in intensive care or with severe forms of the disease (Li, et al., 2020). The mechanisms inducing myocarditis are likely mixed, involving both direct infection of cardiomyocytes and generalized ischemia due to pulmonary impairment and known alveolar-interstitial injuries, with a secondary decrease in gas exchange at this level.

Acute myocarditis can present difficulties in differentiation from myocardial infarction, as the former can show transient ST-segment elevation with spontaneous normalization without intervention. Endomyocardial biopsy can help distinguish between these two etiologies, but it is not feasible during pandemic periods with high patient volumes and overstretched medical services, where transferring patients between departments is difficult. In the direct diagnosis of myocardial dysfunction, computed tomography (CT) offers less information compared to echocardiography and magnetic resonance imaging (MRI); however, it can exclude coronary artery pathology, with a high capacity to rule out calcified atherosclerotic plaques as well as partial or complete occlusions in the coronary system.

Nevertheless, the contribution of CT in COVID-19 patients with myocardial injury was significantly appreciated in the original article of the doctoral thesis, using chest examinations without the injection of contrast material and without a dedicated cardiac protocol. Thus, a prediction model for myocardial involvement was developed, based on biochemical and imaging factors: the extent of pulmonary fibroatelectatic changes, the presence of mediastinal lymphadenopathy, LDH, and D-dimer levels (Dumea, et al., 2022; Ellinghaus, et al., 2020).

3.4. Thrombotic Complications

The hypercoagulable status was observed early in studies during the pandemic in patients with severe forms (Guan, et al., 2020). Frequent changes include elevated levels of D-dimers and fibrinogen concentrations, decreased platelet count, and shortened coagulation time. The association between severe forms and death with venous and arterial thromboses became increasingly stronger as cumulative data grew. Post-mortem analyses revealed microvascular thrombi in the pulmonary, renal, and myocardial beds (Nicolai, et al., 2020). The relationship between complement activation and hypercoagulability has been previously documented and may represent a mechanism favoring frequent thromboses in SARS-CoV-2 infection. Moreover, reduced fibrinolytic capacity in COVID-19 patients has been observed in multiple publications (Bareille, et al., 2021).

Endothelial damage in SARS-CoV-2 infection is a significant triggering factor for thrombus formation, in the context of direct viral infection and the activation of endothelial cells by pro-inflammatory stimuli caused by local chemokines.

Pulmonary embolism (PE) is a significant complication with a high degree of morbidity and mortality associated with the COVID-19 population. The actual incidence of PE and deep vein thrombosis (DVT) is unknown, as some patients suffered from too severe a form to be mobilized to the radiology department. However, a meta-analysis by (Suh, et al., 2021) indicates a presence of PE in 16.5% and DVT in 14.8% among COVID-19 patients. Less than half of the patients with PE also had concurrent DVT, and the emboli's topography was predominantly in peripheral areas. D-dimers showed high sensitivity (96%) but low specificity (10%) for diagnosing PE in this cohort. According to the same research, the incidence of PE was higher among COVID-19 patients compared to patients with other non-COVID severe viral pneumonias admitted to intensive care units, some with associated ARDS.

The hypothesis of in-situ immuno-thrombosis emerged both in the context of peripheral topography and the presence of thrombi in the non-pulmonary arterial bed. Furthermore, the lower prevalence of deep vein thrombosis associated with PE provides additional support for this hypothesis.

3.5. Hepatobiliary Involvement

The presence of chronic liver disease can predispose an individual to an unfavorable outcome during SARS-CoV-2 infection (Marjot, et al., 2021). Patients with liver cirrhosis (of any cause) had a higher mortality rate (32% compared to 8% in non-cirrhotic patients), higher risks of transfer to intensive care units, and a greater need for renal dialysis. Although respiratory symptoms at admission were similar between those with chronic liver disease and those without, gastrointestinal side effects were higher in those with chronic liver disease.

Hepatic involvement in COVID-19 is multifactorial: there is a high expression of ACE2 on cholangiocytes and a low expression on hepatocytes and Kupffer cells. Thus, hepatobiliary complications can be due to direct viral infection at the hepatic level, but also due to exaggerated immune responses, hypoxia, vascular changes and disorders in the context of coagulopathy and endothelial dysfunction, as well as drug-induced liver injury.

The definition of liver injury has varied among authors, focusing on increases in hepatocellular and cholestatic enzymes. According to (Li, et al., 2020), forms of hepatic involvement were classified into specific and non-specific types. Specific types involve a threefold or more increase in ALT/AST levels and a twofold increase in total bilirubin. Non-specific types involve mild and transient increases in liver cytolysis enzymes, not requiring special supportive measures. Severe liver injuries are relatively rare in the COVID-19 population, typically associated with severe forms and multiple organ failure, ARDS, and SIRS (Li, et al., 2020).

3.6. The Role of Imaging in COVID-19 Pathology

Although imaging resource usage protocols varied from country to country, with no unified recommendations, all medical units used imaging as an indispensable tool for diagnosing and monitoring pulmonary involvement, as well as identifying the numerous complications that can arise during the disease.

Chest X-rays are the most accessible and cheapest tool for evaluating the extent of pulmonary involvement in COVID-19 pathology, albeit with a reduced sensitivity (69%) (Wong, et al., 2019). The main findings consist of identifying pulmonary consolidations, nonsystematic and imprecisely delineated opacities. In severe forms, alveolar opacities increase in size and intensity, indicating multilobar, diffuse involvement.

Lung ultrasound and magnetic resonance imaging (MRI) were not frequently used in quantifying and describing pulmonary lesions in COVID-19, given the high costs in terms of human resources and time involved in these examinations. However, both methods are valuable in specific situations, with lung ultrasound having higher sensitivity and specificity rates than chest X-ray in identifying pulmonary lesions (Vetrugno, et al., 2020), and MRI can identify myocarditis, meningoencephalitis, or other types of non-pulmonary complications.

Computed tomography (CT) is by far the most frequently used imaging investigation in evaluating pulmonary involvement in patients with SARS-CoV-2 infection, with a sensitivity of 98% in detecting alveolar-interstitial abnormalities in these patients (Wong, et al., 2019). Typical findings include ground-glass opacities, alveolar consolidations, reticular interstitial thickening (crazy paving), and fibro-atelectatic changes. Involvement was often bilateral, with peripheral and subpleural topography, more frequently in the lower lobes.

Among the complications identified by CT examinations in COVID-19 patients, we include pneumothorax, pneumomediastinum, subcutaneous emphysema, pulmonary thromboembolism, splenic, renal, and mesenteric infarctions (arterial or venous), as well as decompensation of already known pathologies. Lastly, the incidental discovery of neoplasms was another consequence of the high degree of imaging resource utilization.

4. ORIGINAL PART

4.1. Evolution of Imaging Aspects in Patients with Multiple Computed Tomography Examinations

Out of the total patients, 46 (9%) underwent multiple imaging examinations, including 22 women and 24 men.

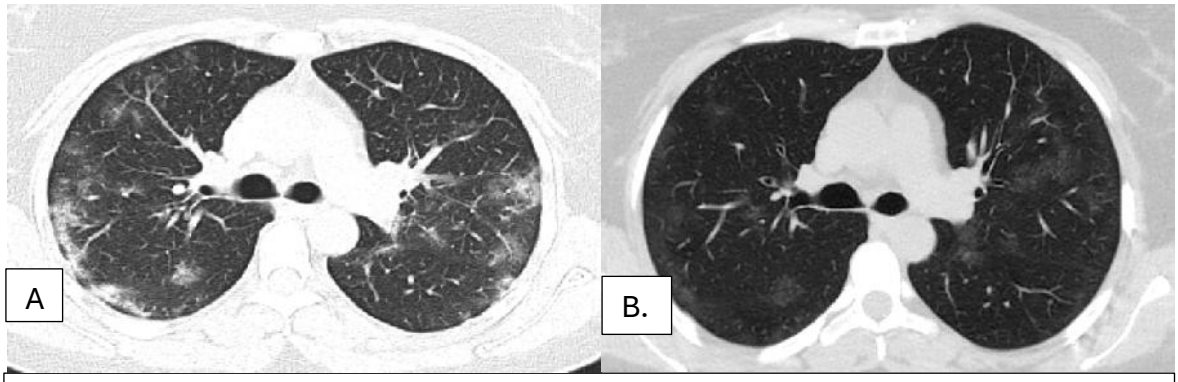

Fig 1. Multifocal "ground glass" areas at diagnosis, involving both lung parenchyma, on the first day of diagnosis (A); The lesions maintain a topography that is not significantly changed, but with a significant regression in intensity, suggesting a remitting aspect of the pneumonia foci, on day 7 (B). *From the archive of INBI "Matei Balş".*

Ground-glass areas represent the most sensitive indicator of the presence of interstitial pneumonia foci in patients with confirmed SARS-CoV-2. Most patients experienced an improvement in visible inflammatory changes on imaging from the first to the second or third examination.

Four stages of imaging evolution of lesions in patients with confirmed SARS-CoV-2 infection are recognized: initial phase (I), within the first 5 days, characterized by multifocal ground-glass areas of variable extent; progression phase (II), up to 8-9 days from onset, with progression of ground-glass areas, diffusely bilaterally distributed, losing the subpleural predilection, including isolated interstitial thickening and alveolar consolidations; peak phase (III), up to 14 days from onset, with extensive alveolar consolidations corresponding to the initial ground-glass areas; resolution phase (IV), with a gradual decrease in intensity of consolidations and ground-glass areas, with some cases showing residual fibrotic changes.

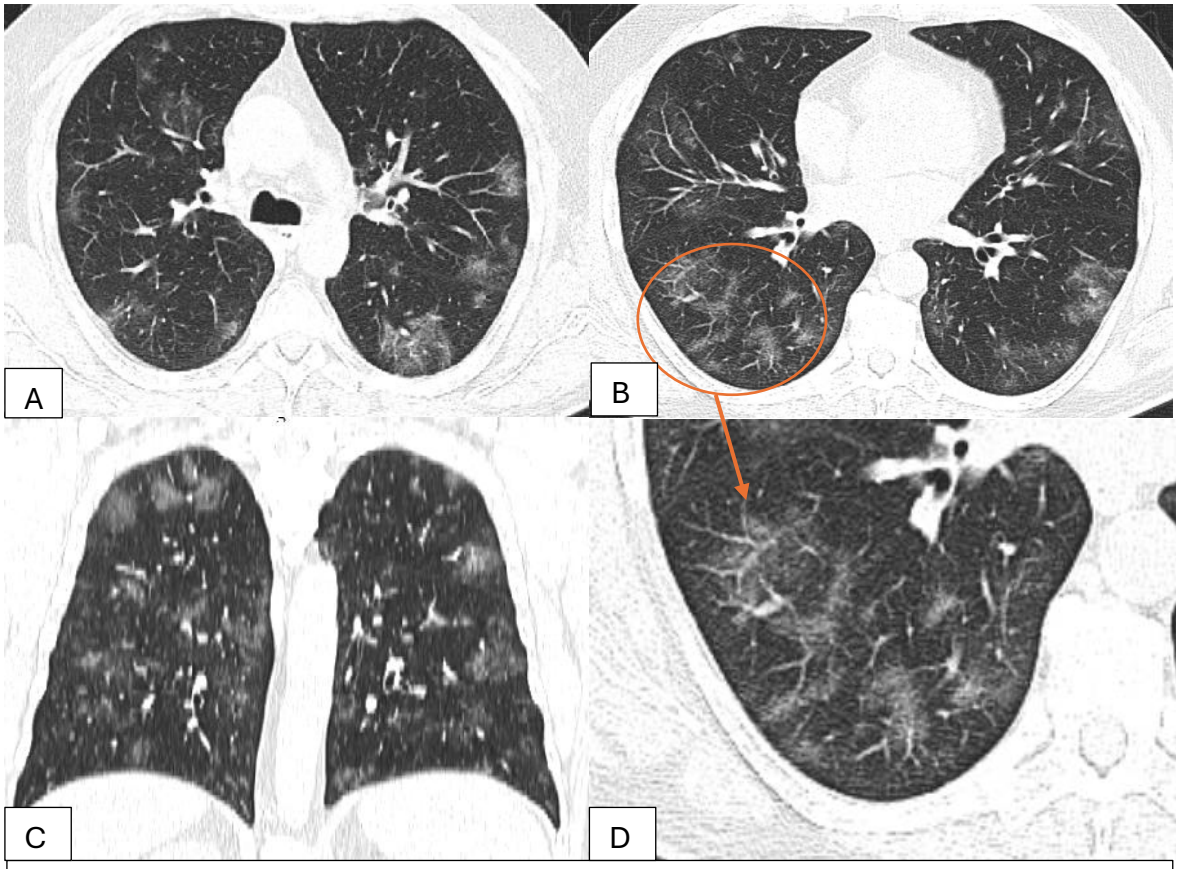

Fig 2. Multifocal "ground glass" areas, some also presenting interlobular septal thickening (of the "crazy paving" type) (B,D). *From the archive of INBI "Matei Bals"*

Regarding the extent of lesions in the pulmonary parenchyma, diffuse involvement was observed in both pulmonary parenchyma, often affecting all lung lobes. No statistically significant differences were found between the degree of extension at the first and the last imaging examination, with improvement being mainly materialized by the reduction in intensity of the lesions and less by their dimensional regression.

Fibro-atelectatic changes were more frequently observed at the second and third imaging examinations, proving their evolutionary nature during SARS-CoV-2 infection.

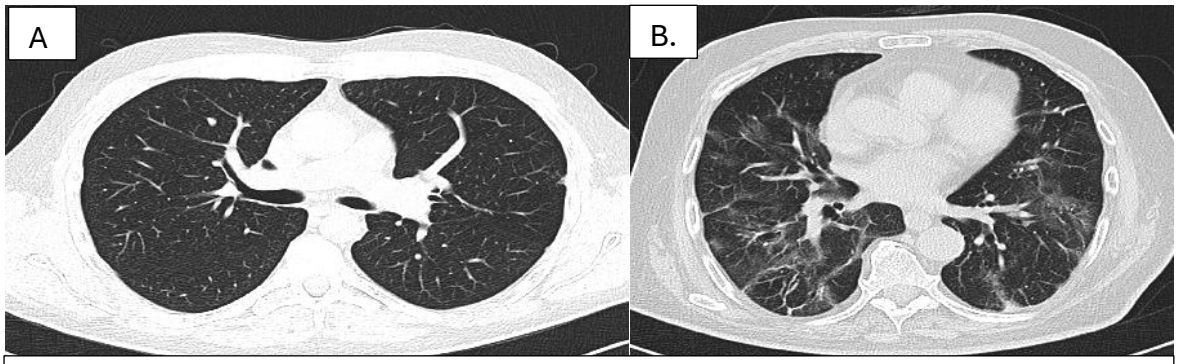

Fig 3. Evolution of fibroatelectatic changes from admission (A) to the third imaging evaluation (B), with a time interval of 21 days between them.*From the archive of INBI "Matei Bals"*

4.2. The extent of pulmonary involvement in COVID-19 patients estimates the risk of myocardial injury

Although SARS-CoV-2 infection primarily affects the lungs, cardiovascular symptoms such as palpitations, fatigue, dyspnea, and chest pain are frequently present in both the acute and chronic stages of the disease. The most common cardiovascular complications are myocarditis, pericarditis, and heart failure.

From the total patient cohort, 150 patients were selected and divided into two groups: patients with biochemical markers indicating myocardial injury (Group A, 18 patients) and patients without biochemical markers of myocardial injury (Group B, 132 patients). The marker for myocardial injury was a serum troponin I value greater than 0.04 ng/mL. The inclusion criteria were those presented in the general methodology of patient selection, and the exclusion criteria added were known endo/myocardial lesions, and chronic cardiac and renal diseases.

Patients with myocardial injury were older, with higher heart and respiratory rates, higher systolic blood pressure, and lower diastolic blood pressure. Statistically significant differences were also identified in TnI, NT-proBNP, LDH, CRP, and D-dimers, with higher values recorded in group A (with myocardial injury present). Serum ferritin, fibrinogen, and IL-6 levels were also higher in group A.

Patients with myocardial injury showed statistically significant differences in terms of the severity of lung disease, the number of lung lobes with pneumonia, interstitial thickening, the extent of fibro-atelectatic changes, and the presence of mediastinal lymphadenopathy. The highest relative risk values for association with myocardial injury were recorded in relation to the presence of mediastinal lymphadenopathy (2.8), the extent of fibro-atelectatic lesions (2.4), interstitial thickening (2.2), the severity of lung disease (2.1), and the number of lung lobes with pneumonia (1.6).

Based on these findings, we performed a multivariable logistic regression model for patients with myocardial injury to identify the most important predictors for myocardial injury, beyond the biochemical marker that defines the lesion. Thus, we identified LDH, D-dimers, the extent of fibro-atelectatic lesions, and the presence of mediastinal lymphadenopathy as optimal parameters for this equation, with an overall prediction percentage of 87.4% and $p<0.001$ (table 1).

Tabel 1 – Multivariable Logistic Regression Model for Patients with Myocardial Injury

Regression Model Pattern	B	S.E.	Wald	p	OR	95% CI for OR	
						Lower	Upper
LDH	0.004	0.003	1.762	0.18	1.004	0.998	1.011
D-dimers	0.001	0.001	3.568	0.05	1.001	1	1.002
Extent of fibroatelectatic lesions	0.881	0.403	4.793	0.02	2.414	1.097	5.313
Presence of mediastinal adenopathy	1.496	0.685	4.778	0.02	4.466	1.167	17.087
Constant	-5.288	1.255	17.761	0	0.005		

Intralobular septal thickening generally suggests lymphocytic infiltration. Therefore, due to direct lung injuries in COVID-19, interstitial pulmonary edema usually indicates an increase in pulmonary vascular permeability, leading to fluid movement into the interstitial compartments (Malek & Soufi, 2023). However, this can also be associated with myocardial injuries, as septal thickening can be induced by a hydrostatic component caused by vasoconstriction, myocardial ischemia, infarction, and arrhythmias (Barile, 2020). A higher incidence of intralobular septal thickening was found in participants from Group A.

Fibroatelectatic lesions, which increase vascular resistance due to lung fibrosis (Kapasi, et al., 2019), were more frequent and severe in Group A, playing an important role in the pathogenesis of cardiac pump dysfunction and secondary myocardial injuries. In pulmonary areas with atelectatic changes, gas exchange tends toward zero, with maintained vascular perfusion and the appearance of an intrapulmonary vascular shunt from right to left. Thus, pulmonary hematosis decreases, aggravating the hypoxia induced by the initial alveolo-interstitial lesion. The hypoxic state and inflammation can play a key role in the pathogenesis of acute myocardial injuries in COVID-19 (Yang, et al., 2020).

The presence of mediastinal lymphadenopathy was more prevalent among participants in Group A. Therefore, we hypothesize that mediastinal lymphadenopathy can be explained in the context of extensive lung lesions and may reveal a bacterial superinfection or an excessive inflammatory response.

Extensive tissue lesions can be indicated by high levels of cytolytic enzymes, including LDH. Although it does not have cardiac specificity, LDH showed a positive correlation with myocardial injuries. This is also evidenced in the literature (Huang, et al.,

2022) (Martha, et al., 2022), which also indicates higher mortality in patients with elevated LDH levels.

D-dimers can be important for diagnosing myocardial injuries and acute coronary syndrome (Reihani, et al., 2018). Our study confirms this, as they are strongly correlated with the presence of myocardial injury. Multiple publications have shown the predictive power of D-dimers for death and poor prognosis in COVID-19 (thromboembolic events, myocardial infarction, renal infarction, ischemic stroke). The main incriminated causes are endothelial dysfunction, disseminated intravascular coagulation, prolonged immobilization, and advanced age.

The main mechanisms of myocardial injury in COVID-19 reported include excessive inflammation and cytokine-mediated injuries, direct myocardial invasion by the virus, the imbalance between oxygen supply and demand under respiratory failure and hypoxemia conditions, hypercoagulability and the presence of endothelial inflammation, as well as impairment of coronary microvascular flow due to thrombus formation or plaque rupture with secondary myocardial ischemia (Bavishi, et al., 2020).

4.3. Clinical, Biochemical, and Pulmonary Imaging Characteristics in Hepatobiliary Involvement of COVID-19 Patients

Hepatobiliary involvement during SARS-CoV-2 infection is significant, considering the liver's role in overall homeostasis (metabolism, coagulation, detoxification, immune response to infections, and protein synthesis in general). Thus, liver injury can be associated with systemic changes that may require adjustments in the management of COVID-19 patients.

SARS-CoV-2 can infect and replicate in liver cells, leading to inflammation and fibrosis (Wang, et al., 2020). Elevated levels of hepatic cytolysis enzymes were found both in our study and in the literature (Zhou, et al., 2020).

The inclusion criteria were the same as those presented in the general methodology chapter, and the exclusion criteria added to the list patients with known liver diseases before hospitalization (viral, autoimmune, toxic hepatopathies) and chronic kidney disease, as well as those receiving hepatotoxic treatment.

We defined hepatobiliary involvement as an increase in serum levels of ALT, AST, total and direct bilirubin, and GGT. Subsequently, we defined the hepatocellular injury syndrome as a strict increase in ALT and AST, the cholestasis syndrome as a strict increase

in total bilirubin and GGT, and mixed involvement as an increase in parameters from both subgroups. Based on these definitions, two groups were formed: with (Group A) and without (Group B) hepatobiliary involvement, further defining three subgroups according to the type of involvement: A1 (hepatocellular injury), A2 (cholestasis), A3 (mixed).

The total cohort consisted of 132 patients, of whom 95 (72%) presented hepatobiliary involvement. Among these, 26 formed subgroup A1, 12 in subgroup A2, and 57 patients in subgroup A3. Patients with any type of hepatobiliary involvement had a lower oxygen saturation rate (92% vs 97%) and a longer hospitalization period (12 days vs 7 days, $p<0.05$), with a predominance of male patients.

Serum ferritin was elevated among patients with hepatobiliary involvement, while serum albumin was lower among them, with total protein levels remaining within normal limits. Although CRP and D-dimers recorded higher levels in Group A, they did not show statistically significant differences between the two groups.

ANOVA analysis of the subgroups (A1, A2, A3) identified statistically significant differences in biochemical markers. Serum ferritin and C-reactive protein levels were higher in the cholestasis and mixed involvement subgroups (A2 and A3). Atelectatic changes were more prevalent in the mixed involvement subgroup (A3).

In this study, we found that a large percentage of patients with SARS-CoV-2 infection exhibit hepatobiliary involvement (72%), consistent with literature results (Fan, et al., 2020). The more frequent types of involvement were cholestatic and mixed, which were associated with higher serum ferritin levels, while the mixed subgroup had higher D-dimer levels compared to the other subgroups. Additionally, positive correlations with CRP levels were found, indicating the importance of systemic inflammation in the context of hepatobiliary involvement, in line with literature reports (Shen, et al., 2021), especially since these increases were also reported in myocardial and pericardial involvement (Lazar, et al., 2022).

Reduced albumin levels were also reported, although with slightly modified percentages (4% (Guan, et al., 2020) compared to 9% in our study). The data indicate an association between reduced albumin levels and severe forms of pneumonia, longer hospitalization periods, and increased mortality. However, low albumin levels should not correspond to reduced liver function, as albumin is a negative acute phase reactant: the prioritization of the synthesis of other acute phase proteins over albumin represents a necessary physiological adaptation. Moreover, considering the recognized gastrointestinal and renal involvements of SARS-CoV-2, albumin loss can also occur enterally (during diarrheal episodes caused by infection) and renally when the glomerulus is affected.

The conducted study is valuable due to its selection characteristics, with exclusion criteria eliminating patients known to have previous liver conditions (viral, autoimmune, toxic) as well as those on hepatotoxic medication. Stratified representation and the finding of statistically significant differences between types of hepatobiliary involvement are other valuable points of the research work.

4.4. COVID-19 Associated Pulmonary Thromboembolism: Clinical, Biochemical, and Imaging Findings

One of the most severe complications in patients with SARS-CoV-2 infection, pulmonary thromboembolism, must be identified and treated as early as possible, with sectional imaging and biochemical profiles being particularly important in this context.

According to literature data, males, obesity, mechanical ventilation, severe forms of pneumonia, transfer to the intensive care unit, elevated D-dimer levels, and leukocytosis are associated with a higher risk of pulmonary thromboembolism in COVID-19 patients (Cui, et al., 2021). The actual incidence of this complication is difficult to estimate precisely, given that a significant number of patients cannot benefit from imaging diagnosis, either due to logistical reasons or the inability to be mobilized due to severe forms of the disease.

The inclusion criteria were those presented in the general methodology of patient selection, while the exclusion criteria included patients with chronic pulmonary embolism, chronic heart and/or kidney disease, and known antiphospholipid syndrome. Additionally, those receiving anticoagulant treatment for pre-existing conditions were also excluded.

In addition to the usual imaging data selected, the characterization of thrombi in patients with pulmonary thromboembolism (PTE) included: the number of pulmonary segments affected by PTE, the extension into the main pulmonary artery or the trunk of the pulmonary artery, and the density of the lung parenchyma adjacent to the thrombus.

Patients with SARS-CoV-2 infection (186) were divided into two groups: Group A consisted of patients without PTE (155 patients), and Group B consisted of patients with filling defects in the arterial tree observed on imaging (31 patients). Patients in Group B were older, predominantly male, had longer hospitalization periods, and had a higher mortality rate compared to the group of patients without PTE.

Ferritin, myoglobin, LDH, CRP, and IL-6 levels were higher in Group B (the group with pulmonary thromboembolism) with a p-value of less than 0.05. Serum albumin and

total protein levels, as well as lymphocyte counts, were lower in Group B. Contrast-enhanced computed tomography (CT) scans at admission revealed statistically significant differences in almost all imaging characteristics and scales used, with a greater extent of ground-glass opacities; alveolar consolidation; interstitial thickening with a "crazy paving" pattern; vascular ectasias, bronchiectasis, and fibro-atelectatic changes.

Thrombi were predominantly located in the segments of the lower lobes, with a slight preference for the right side. The majority presented with unilateral pulmonary embolism (61.3%) and with segmental and subsegmental localization. The average densitometric measurements of the lung parenchyma adjacent to the embolized vascular bed suggest that pulmonary embolism predominantly occurred in pulmonary segments with previous pneumonia involvement.

Positive correlations with the presence of an acute thrombus in the pulmonary arterial tree were obtained with LDH, ferritin, IL-6, leukocyte count, and D-dimer levels ($p<0.001$). Additionally, ROC curve analysis identified D-dimers, LDH, IL-6, serum total proteins, serum ferritin, duration of hospitalization, leukocyte count, and myoglobin as positive predictive factors for pulmonary thromboembolism (PTE). From an imaging perspective, the extent of ground-glass opacities, interstitial thickening, alveolar consolidation, and the presence of vascular ectasias showed statistically significant correlations with the presence of PTE. The best predictors according to the area under the curve (AUC) were D-dimers (0.921) and ground-glass opacities (0.919).

The multivariable logistic regression model identified the following elements with high predictive value for the presence of PTE, recording an overall prediction percentage of 90.7% with $p<0.001$: LDH, serum ferritin, IL-6, and the extent of alveolar consolidations.

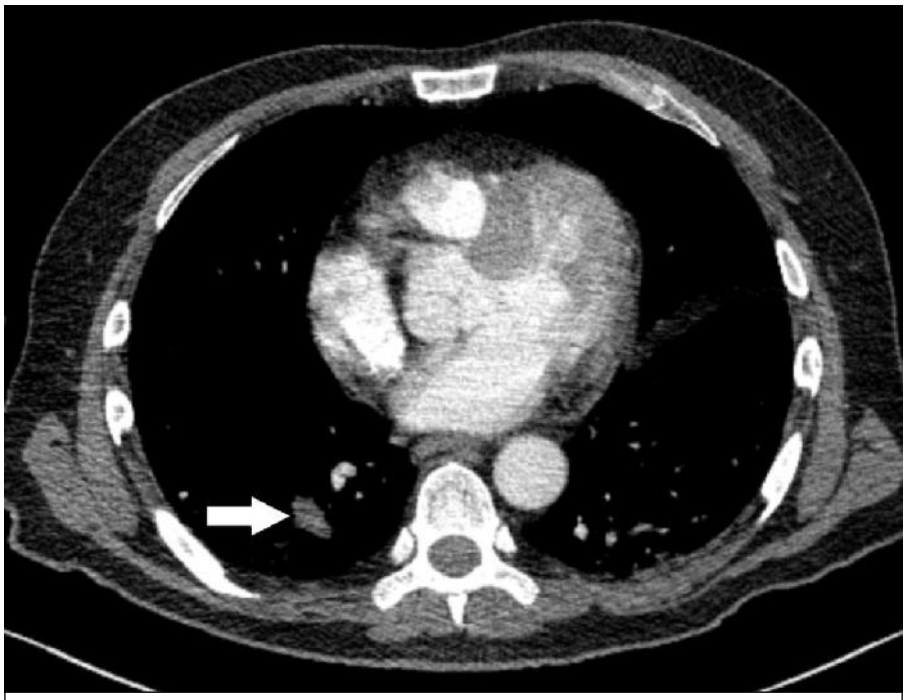

Fig 4. Right lower lobar pulmonary artery with enlarged caliber and completely thrombosed, with the absence of endoluminal contrast visualization at this level (arrow). *From the archive of INBI "Matei Bals"*

Thus, patients with pulmonary thromboembolism (PTE) in the study were older, predominantly male, with longer hospitalization periods and increased mortality. They presented more severe forms of the disease, evidenced by statistically significant differences in both the biological and imaging panels. CRP, LDH, serum ferritin, IL-6, serum myoglobin, NT-proBNP, D-dimers, serum albumin, and total serum proteins showed statistically significant differences among patients with pulmonary embolism. The extent of lung lesions was also more extensive, with diffuse interstitial thickening, vascular ectasias, and prominent fibroatelectatic changes.

These findings are validated by specialized publications, especially those using collaborative national databases (Gul, et al., 2023), which found both higher mortality in patients with PTE and the value of D-dimers, LDH, and serum ferritin in identifying patients with pulmonary embolism. Similarly, they identified age and male gender as risk factors for developing these thrombotic complications. However, the literature continues to be heterogeneous, with some studies failing to identify correlations between inflammatory markers and the presence of PTE (Yousaf, et al., 2023) or differences in mortality (Riyahi, et al., 2021). This heterogeneity of results between studies can be at least attributed to different selection criteria and populations, as well as different strains and evaluation periods from one study to another.

5. Conclusions and Personal Contributions

The patterns of pulmonary involvement during SARS-CoV-2 infection are important to recognize, allowing dynamic evaluation of the disease's course both clinically-biologically and through imaging. Given the volume of imaging investigations that COVID-19 patients have undergone, the main patterns of involvement visible on CT scans were identified, as well as their progression and improvement.

Ground-glass areas represent the most sensitive imaging manifestation seen in patients with confirmed SARS-CoV-2 infection, which can evolve into alveolar consolidations with associated interstitial thickening and latent fibroatelectatic changes corresponding to the initial areas of involvement. The way these lesions evolve or improve can change the treatment course that patients follow.

Although imaging investigations were performed to evaluate the lung parenchyma during the infection, additional, non-apparent information could be extracted from them. Knowing that SARS-CoV-2 infection affects not only the lung parenchyma but also other organs and systems, we were able to evaluate the correlations of chest imaging with the clinical-biological profile of non-pulmonary involvement.

Thus, the research focused not only on presenting the imaging aspects of lung parenchyma involvement but also on calculating the risks of myocardial injury, hepatobiliary involvement, and thromboembolic events based on the recorded imaging patterns, along with the observed biochemical changes.

We showed correlations between the degree and patterns of lung involvement and the risk of myocardial injury, indicating the importance of monitoring patients not only for respiratory manifestations but also for potential cardiovascular complications.

Systemic inflammation and especially endothelial dysfunction predispose patients to thromboembolic events, with pulmonary thromboembolism being among the most well-known and severe complications. We recorded statistically significant differences in multiple biochemical and imaging parameters between patients with and without pulmonary thromboembolism, indicating a globally more severe disease with notable changes between the mentioned panels in patients with embolic manifestations.

Hepatobiliary involvement represents an important aspect of COVID-19 patients, being associated with longer hospitalization periods, higher levels of inflammation markers, and prolonged coagulation parameters. Hepatobiliary involvement and impairment can alter

the therapeutic plans for patients with SARS-CoV-2 infection, as many medications can exacerbate liver dysfunction.

The creation of risk prediction models for myocardial injury and the presence of pulmonary thromboembolism presents novel aspects of this thesis. Up to the time of publishing, no other scientific works were identified that addressed these topics in this manner. The stratification of hepatobiliary involvement types is another novelty, providing a more accurate characterization of the complex manifestations at this level.

Bibliography

Zhang, et al., 2020. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19.. *Science,* 370(eabd4570).

Jacobs, et al., 2020. Increased expression of ACE2, the SARS-CoV-2 entry receptor, in alveolar and bronchial epithelium of smokers and COPD subjects. Volume 56.

Alqahtani, et al., 2020. Prevalence, severity and mortality associated with COPD and smoking in patients with COVID-19: a rapid systematic review and meta-analysis. *PloS one,* 15(e0233147).

Zhang, et al., 2020. Histopathologic changes and SARS-CoV-2 immunostaining in the lung of a patient with COVID-19. *Annals of internal medicine,* 172(629-632).

Chen, Zhou & Dong, 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet,* 395(507-513).

Guan, Ni, Z. & Hu, Y., 2020. China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. *NEJM,* 382(1708-1720).

Hanff, et al., 2020. Is there an association between COVID-19 mortality and the renin-angiotensin system-a call for epidemiologic investigations. *Clinical Infectious Diseases,* 71(870-874).

Li, Yang. & Zhao, 2020. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clinical research in cardiology,* 109(531-538).

Dumea, E. et al., 2022. Pulmonary Involvement in SARS-CoV-2 Infection Estimates Myocardial Injury Risk.. *Medicina,* 58(10), p. 1436.

Ellinghaus, et al., 2020. Genomewide association study of severe Covid-19 with respiratory failure.. *NEJM,* 383(1522–34).

Guan, et al., 2020. Clinical characteristics of coronavirus disease 2019 in China.. *NEJM,* Volume 382:1708–20.

Nicolai, et al., 2020. Immunothrombotic dysregulation in COVID-19 pneumonia is associated with respiratory failure and coagulopathy. *Circulation,* Volume 142, pp. 1176-1189.

Bareille, et al., 2021. Viscoelastometric testing to assess hemostasis of COVID-19: a systematic review. *Journal of clinical medicine,* Volume 10:81740.

Suh, Y. J., Hong, H., Ohana, M. & Bompard, F., 2021. Pulmonary Embolism and Deep Vein Thrombosis in COVID-19: A Systematic Review and Meta-Analysis. *Radiology,* Volume 298:2, E70-E80.

Marjot, et al., 2021. Outcomes following SARS-CoV-2 infection in patients with chronic liver disease: an international registry study.. *Journal of Hepatology,* Volume 74: 567–577.

Li, SY & Xiao, 2020. Hepatic involvement in COVID-19 patients: pathology, pathogenesis, and clinical implications.. *Journal of medical virology,* Volume 92: 1491–1494.

Wong, et al., 2019. Frequency and distribution of chest radiographic findings in COVID-19 positive patients. *Radiology,* Volume 27:201160.

Vetrugno, et al., 2020. Our Italian experience using lung ultrasound for identification, grading and serial follow-up of severity of lung involvement for management of patients with COVID-19. *Echocardiography,,* 37(4), pp. 625-7.

Malek & Soufi, 2023. Pulmonary Edema. *StatPearls.*

Barile, M., 2020. Pulmonary Edema: A Pictorial Review of Imaging Manifestations and Current Understanding of Mechanisms of Disease.. *European journal of radiology open.*

Kapasi, A. et al., 2019. Elevated pulmonary vascular resistance is associated with increased risk of death in IPF. *Eur. Respir. J.* .

Yang, L. et al., 2020. Hypoxia and inflammation are risk factors for acute myocardial injury in patients with coronavirus disease 2019.. *Beijing Da Xue Xue Bao Yi Xue Ban,* Volume 53, pp. 159-166.
Huang, Y. et al., 2022. Serum Lactate Dehydrogenase Level as a Prognostic Factor for COVID-19: A Retrospective Study Based on a Large Sample Size.. *Front. Med.,* Volume 8.
Martha, J., Wibowo, A. & Pranata, 2022. Prognostic value of elevated lactate dehydrogenase in patients with COVID-19: A systematic review and meta-analysis.. *Postgrad. Med. J.,* Volume 98, pp. 422-427.
Reihani, H., Shamloo, A. & Keshmiri, 2018. Diagnostic Value of D-Dimer in Acute Myocardial Infarction Among Patients With Suspected Acute Coronary Syndrome.. *Cardiol. Res.,* Volume 9, pp. 17-21.
Bavishi, C. et al., 2020. Acute myocardial injury in patients hospitalized with COVID-19 infection: A review.. *Prog. Cardiovasc. Dis.,* Volume 63, pp. 682-689.
Wang, et al., 2020. COVID-19 and the liver: a systematic review.. *J Med Virol. ,* Volume 92, pp. 1289-95.
Zhou, et al., 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study.. Volume 395:1054–62.
Fan, Chen & Li, 2020. Clinical features of COVID-19-related liver functional abnormality.. *Clin Gastroenterol Hepatol.,* Volume 18, pp. 1561-6.
Shen, Zhuang & Zhang, 2021. Risk factors and prognosis in patients with COVID-19 and liver injury: a retrospective analysis.. *J Multidiscip Healthc. ,* Volume 14, pp. 629-37.
Lazar, M. et al., 2022. Pericardial Involvement in Severe COVID-19 Patients. *Medicina,* Volume 58, p. 1093.
Cui, et al., 2021. Risk factors for pulmonary embolism in patients with COVID-19: a systemic review and meta-analysis. *nternational Journal of Infectious Diseases,* Volume 111, pp. 154-163.
Gul, et al., 2023. Predictors and outcomes of acute pulmonary embolism in COVID-19; insights from US National COVID cohort collaborative.. *Respiratory Research,* Volume 59.
Yousaf, Thomas, Almughalles & Hameed, 2023. Pulmonary embolism in COVID-19, risk factors and association with inflammatory biomarkers. *Medicine,* 102(7), p. e32887.
Riyahi, et al., 2021. Pulmonary embolism in hospitalized patients with COVID-19: a multicenter study.. *Radiology.*