UNIVERSITY OF MEDICINE AND PHARMACY

„CAROL DAVILA", BUCHAREST

DOCTORAL SCHOOL

MEDICINE

Clinical and imaging evolutionary aspects in moderate and severe SARS-CoV-2 infection

ABSTRACT OF DOCTORAL THESIS

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2023

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I. GENERAL PART

In the first chapter of this part we have described in detail the microbiological and epidemiological characteristics of SARS-CoV-2, which was first identified in December 2019 in China and causes the disease called COVID-19 (Delorey et al., 2021). Since being declared a pandemic by the WHO on March 11, 2020, SARS-CoV-2 infection has been diagnosed in over 600 million patients globally and has caused over 6 million deaths, with at least 60,000 reported in our country (Tuculeanu et al., 2023). We have also illustrated, using special medical software, elements of general pathogenesis in COVID-19, supporting its severe evolutionary potential and progression to ARDS, through the emergence of a true "cytokine storm".

The second chapter included an extensive literature review supporting the complex pathophysiological mechanisms leading to different clinical pictures compatible with lung damage in the context of COVID-19. These are based on a mixed component, both secondary to viral parenchymal lesions and immunothrombotic, in the form of *in situ* pulmonary artery thrombosis secondary to excessive inflammation at the pulmonary and systemic level. COVID-19-associated pulmonary thrombotic events actually represent a pathophysiological outcome of common pathways of immune mediation, endothelial dysfunction, coagulopathy and platelet dysfunction, in association with classical risk factors for thromboembolic disease and probably other unknown mechanisms, each contributing to some degree to their occurrence, difficult to assess (Loo et al., 2021, Conway et al., 2022, Portier et al., 2021, Niculae et al., 2023a, Niculae et al., 2023b).

We have made an extensive synthesis that included the pathophysiological elements that "orchestrate" this complex process of thrombosis, both inflammatory and coagulation. Inflammation pathways include humoral and cellular mediation, represented mainly by macrophages, monocytes, T lymphocytes, mast cells, cytokines (IL-6 with a central role), chemokines, the complement system, but also the NETs-thrombosis molecular axis (Niculae et al., 2023b). The biological pathways of the coagulopathy system involve intrinsic and extrinsic pathways, altered fibrinolysis balance (central role of PAI-1), but also endothelial cells, platelets, erythrocytes, von Willebrand factor, thrombomodulin, P-selectin (Niculae et al., 2023b).

II. PERSONAL CONTRIBUTIONS

Chapter 3. Aim and objectives

With this research I aimed to optimize the identification of factors associated with unfavorable outcomes of disease, but also treatment and also to evaluate the clinical response of patients hospitalized with COVID-19 pneumonia by integrating clinical, biological and quantitative lung CT imaging elements. To achieve this goal, we formulated five objectives, which were addressed in five original studies and two literature review articles.

The general objectives of this scientific work, in the context of the definition of the scope, were:

1. Exploring the prothrombotic status associated with excessive inflammation in COVID-19 and its impact on patient prognosis

1.1 Description of imaging features in the context of defining the term *in situ* pulmonary immunothrombosis and quantifying the prognosis of patients with pulmonary thrombosis (PT) associated with SARS-CoV-2 infection.

1.2 To highlight the excessive prothrombotic status associated with severe forms of COVID-19 as part of the general pathophysiological process supporting immunothrombosis, by describing the clinico-imaging characteristics of patients with a diagnosis of PT developed as a result of anticoagulation failure.

2. To study the impact of treatment with corticosteroids and tocilizumab (TOC) by describing the imaging evolution in patients hospitalized with COVID-19 pneumonia and treated with CS plus TOC and assessing the optimal timing of TOC administration in relation to oxygen flow and correlation with impact on in-hospital mortality

3. Quantification of risk factors for specific clinical features during SARS-CoV-2 infection

3.1 Identify risk factors for pericardial fluid effusion and their impact on patient prognosis.

3.2 Analysis of mortality risk factors in SARS-CoV-2 infection with integration of quantitative CT imaging into a prognostic score.

Chapter 4. General research methodology

The common general methodology of this PhD work was based on observational, cohort, analytical sub-studies including adult patients (over 18 years) hospitalized with SARS-CoV-2 infection confirmed by RT-PCR or Ag SARS-CoV-2 at the National Institute of Infectious Diseases "Prof. Dr. Matei Bals", Bucharest.

Their enrolment period was from the onset of the COVID-19 pandemic, i.e. from March 2020, when the first patients infected with the new coronavirus were admitted to our country and until June 2022, when the hospital was no longer dedicated exclusively to the care of these patients. General demographic, clinical, biological and imaging data were collected through a dual methodology, both retrospectively, for the period March 2020-December 2021, and prospectively, December 2021-June 2022. We excluded patients with insufficient data for statistical analysis or those without CT imaging. The definition of the clinical form of SARS-CoV-2 infection was according to literature data at the time of the studies (Anghel et al., 2022, Mehta et al., 2020).

Particularly important was the support of the Department of Radiology and Medical Imaging of the National Institute of Infectious Diseases "Prof. Dr. Matei Bals", and the close collaboration with the coordinator of the department, Conf. Univ. Dr. Mihai Lazar, especially for the methodology of CT image analysis and the specific protocols for each study.

Lung imaging examinations were performed in our clinic on a 64-slice *Somatom Definition As* (Siemens) CT scanner. The percentage of lung parenchymal damage was calculated based on density intervals using a dedicated software model *Syngo Pulmo3D* (Siemens Healthcare GmbH, Erlangen, Germany), which allowed us to segment the lung parenchyma (excluding the main pulmonary vessels, trachea and main bronchi from the densitometric assessment) and calculate the percentage of lung damage based on specific densitometric intervals.

Thus, alveolar lung lesions (consolidation) were considered for densities > 0 HU, mixed lesions between 0 and -200 HU, interstitial lesions between -200 and -800 HU, normal lung densities between -800 and -1000 HU, and hyperinflation or emphysema for densities less than -1000 HU (Anghel et al., 2022). For the diagnosis of pulmonary thrombosis the standard protocol included CT pulmonary angiography (CTPA).

Data for statistical analysis were processed using SPSS**.**

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Chapter 5. Clinical and imaging features of thrombotic pulmonary vascular damage in moderate and severe COVID-19

In this chapter we have achieved the number one objective of this PhD work, to explore the prothrombotic status associated with excessive inflammation in COVID-19 and its impact on patient prognosis. The results were presented in the form of two original studies and two extended literature review articles, supporting the clinical data from our studies and explaining, in immunological and pathophysiological details, the mechanisms of pulmonary *in situ* immunothrombosis, indirectly evidenced by both the imaging aspects presented and the failure of anticoagulation.

Objective 1.1. To describe imaging features in the context of defining the term *in situ* pulmonary immunothrombosis and quantify the prognosis of patients with PT associated with SARS-CoV-2 infection

This objective was achieved in the first study presented in the PhD thesis, namely "*Quantitative lung CT imaging features and prognosis of patients with COVID-19 associated pulmonary artery thrombosis*".

Introduction

For this study we started from the idea of the pathophysiological peculiarity of pulmonary thromboses associated with SARS-CoV-2 infection, namely their localization in anatomical areas with inflammation (pulmonary immunothrombosis *in situ*) and/or in the form of microthromboses, with diffuse occlusive thrombotic microangiopathy associated with alveolar lesions (Manolis et al., 2021, Mueller-Peltzer et al., 2020).

Materials and methods

This sub-study involved a mixed retrospective (March 2020-December 2021) and prospective (December 2021-June 2022) enrolment of a cohort of patients hospitalized with COVID-19 who underwent CTPA for suspected PT.

We only included patients who had imaging performed in our clinic. We excluded patients who had poor contrast uptake in the pulmonary arteries and those whose images showed significant motion/breathing artefacts that did not allow good image analysis.

We used the terminology of PT to cover both pathophysiological mechanisms potentially involved in macrovascular thrombotic events in patients with COVID-19, i.e. pulmonary thromboembolism and immunothrombosis *in situ* (Conway et al., 2022, Loo et al., 2021).

Results - Characteristics and prognosis of patients diagnosed with pulmonary artery thrombosis

For this analysis we included 73 patients, which we divided into two groups: group A (36 patients with pulmonary artery thrombosis) and group B (37 patients without pulmonary artery thrombosis). All patients had severe forms of COVID-19.

In terms of comparative results, although the two groups of patients with SARS-CoV-2 infection were similar in terms of age, gender, comorbidities, clinical picture, Wells score and anticoagulant treatment, patients in group B without a diagnosis of COVID-19-associated pulmonary artery thrombosis had a higher risk of developing this thrombotic pathology according to the PADUA prediction score (4.6 vs. 5.5, p = .01). Only one patient in the PT group had clinical signs of DVT. Except for the D-dimers (median of 3142 vs. 533, p = .002), coagulopathy and markers of inflammation were not statistically significantly different between the two groups.

ROC curve analysis of D-dimer showed that a D-dimer value above 1716 ng/mL can predict the diagnosis of PT with an AUC of 0.779, 72.2% sensitivity and 73% specificity (95% CI 0.672-0.885).

The number of lung lobes/segments analyzed by quantitative CT imaging with parenchymal inflammatory parenchymal interstitial/alveolar involvement was also similar in patients with and without COVID-19 associated PT. Logistic regression statistical analysis showed that only increased D-dimer values were associated with the diagnosis of PT (p = .012). Other variables analyzed were sex (p = .3), age (p = .9), presence of comorbidities (p = .6), CRP (p = .7), ferritin (p = .3), IL-6 (p = .3), and presence of total lung injury (p = .5). PT was associated with the need for mechanical ventilation and ICU admission (30.5% vs. 8.1%, p = .01), but not with statistically significantly higher all-cause in-hospital mortality (22.2% vs. 18.9%, p = .7).

Results - Imaging characteristics of patients diagnosed with pulmonary artery thrombosis

Most patients had peripheral, segmental/subsegmental PT. The distribution of TP and total inflammatory lung lesions is shown in the figure below. We can notice a particular involvement of the lower compared to the upper lobes, consistent with the higher prevalence of PT in the lower lobes.

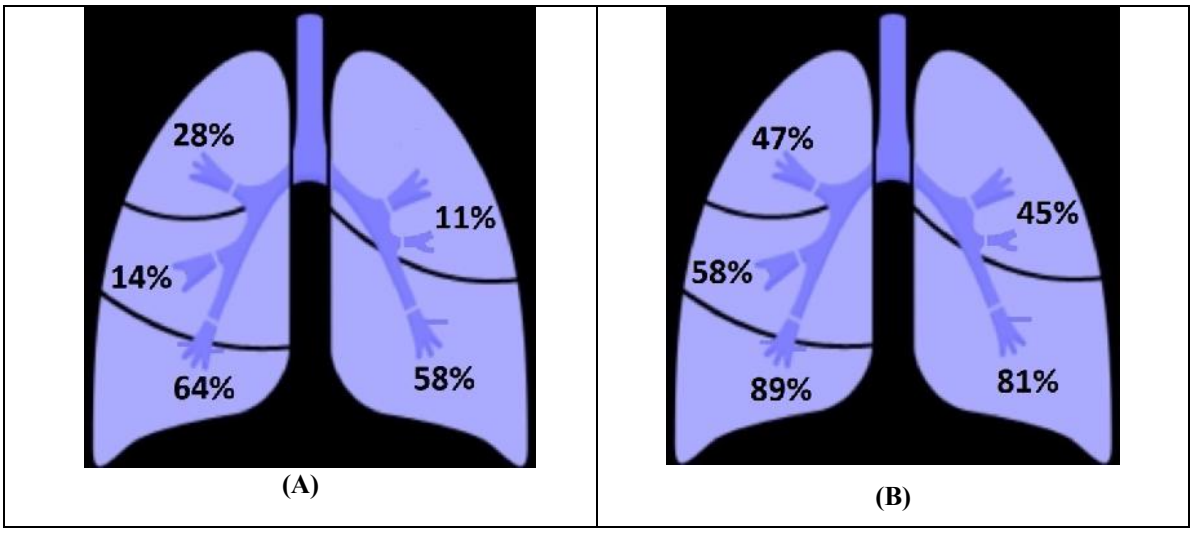


Figure 5.1. Distribution of pulmonary artery thrombosis in the study group (A); percentage and distribution of inflammatory lesions (B)

Although patients with pulmonary thrombosis had a median percentage of parenchyma without inflammatory damage of 32.4%, in 91.6% of cases the presence of thrombi was detected in parenchyma with alveolar or interstitial lesions.

Conclusions

Diffuse thrombosis and its localization mainly in the lung anatomical areas with inflammation, i.e. in the bases and involving mainly peripheral, segmental and subsegmental arteries, suggests a possible association between vascular thrombosis and viral inflammatory lesions, framing this pathophysiological concept of pulmonary immunothrombosis *in situ*. The lack of classical thrombosis risk factors and clinical signs of DVT further support this clinical hypothesis at the expense of the embolic mechanism.

The results of our study showed no statistically significant difference in in-hospital mortality between patients with and without PT, but it was associated with the need for mechanical ventilation and ICU admission.

Objective 1.2 To highlight the excessive prothrombotic status associated with severe forms of COVID-19 as part of the overall pathophysiological process supporting immunothrombosis, by describing the clinical-imaging characteristics of patients with a diagnosis of PT, developed as a result of anticoagulation failure.

This objective was achieved in the second study presented in the PhD thesis, namely "*Anticoagulation failure in SARS-CoV-2 infection: acute pulmonary thrombosis developed under anticoagulant therapy in patients with COVID-19 pneumonia* ", accompanied by an extensive literature review supporting the clinical observations. It also included a presentation of a particular clinical case.

Introduction

Anticoagulation failure in thrombotic disease is a recognized problem particularly in patients with active neoplastic disease (Horne, 2001, Mosarla et al., 2019), but not well studied in SARS-CoV-2 infection.

Thrombotic pulmonary events may occur in patients with COVID-19 and associated pneumonia despite prophylactic anticoagulation (Lim and McRae, 2021, El-Qutob et al., 2022, Kutsogiannis et al., 2022, Mazloomzadeh et al., 2021, Klok et al., 2020). However, there is no consensus on the optimal anticoagulation strategy (Chandra et al., 2022, Porfidia and Pola, 2020).

Materials and methods

This was an observational, retrospective cohort sub-study including patients admitted with COVID-19 between March 2020 and December 2021. The definition of disease severity followed the general methodology described.

We included adult patients with a diagnosis of PT who received anticoagulant therapy with LMWHs (prophylactic, intermediate or therapeutic doses) for ≥ 72 hours until diagnosis of PT by CTPA. Of all patients hospitalized and retrospectively reviewed, 67 were screened for eligibility.

We excluded patients with (a) acute renal failure or chronic kidney disease with a creatinine clearance ≤ 30 ml/min (2 patients) and (b) confirmed or suspected DVT on admission (3 patients) and (c) high suspicion of PT on admission (Wells score ≥ 2) and D-dimer ≥ 1000 ng/mL) without a previous CTPA for PT exclusion (27 patients) and (d) chronic PT or an episode of PT within the last six months (5 patients).

Doses of LMWHs have been grouped as prophylactic or therapeutic doses based on their summary of product characteristics. For patients with a BMI ≥ 40 kg/m^2 , enoxaparin as 40 mg subcutaneously twice daily and dalteparin up to 6500 units once daily were considered to be prophylactic doses(Nutescu et al., 2009). Intermediate LMWHs regimens were defined for enoxaparin as 40 mg subcutaneously twice daily up to 0.5 mg/kgc, according to the HEP-COVID study (Spyropoulos et al., 2021), but we considered as intermediate any dose between a prophylactic or full dose.

We calculated Wells and PADUA scores according to a detailed methodology separately.

Results - Clinical, biological and imaging characteristics of patients diagnosed with PT under different anticoagulation regimens

During the study period, 30 patients were hospitalized with COVID-19 pneumonia. The median age was 62 (54-74) years, with 83.3% male, and comorbidities were observed in 73.3% of patients, the most common being obesity, type 2 diabetes mellitus and cardiovascular comorbidities.

Given the PADUA score, 86.6% of patients had a high risk of developing VTE, but 80% of patients studied had a low probability of a pulmonary embolism diagnosis according to the Wells score calculated and validated for PTE, not for PT in general.

Also, none of our patients had clinical signs of DVT at diagnosis. We also noted the absence of classic thrombosis factors in most patients. We found more than 50% pulmonary involvement in 73.3% of patients with COVID-19 on chest CT at admission.

PT was diagnosed despite prophylactic, intermediate or therapeutic doses of LMWHs for an average of 8 (4.7-12) days after hospital admission. None of the anticoagulated patients had anti-factor X_a levels monitored during treatment with LMWHs.

Next, we compared clinical and laboratory data between the time of hospital admission and the diagnosis of PT (Table 5.4). At the diagnosis of PT we found a worsening of the respiratory function, with seven patients progressing to MV (p=.006).

Table 5.4. Clinical and laboratory data on admission and at the time of PT diagnosis.

Variables	At Hospital Admission (n = 30)	At PT Diagnosis (n = 30)	*p* Value
Oxygen flow rate, N (%)			
≤15 (L/min)	12 (40)	5 (16.6)	.006
HFOT	13 (43.3)	13 (43.3)	-
Mechanical ventilation	5 (16.6)	12 (40)	.006
Leukocyte count (cells/mm^3), mean ± SD	8430 ± 3520	12,105 ± 4795	.001
Lymphocyte count (cells/mm^3), mean ± SD	800 ± 456	959 ± 545	.1
Neutrophils/lymphocytes ratio, median (25–75th percentile)	8.8 (6.3–14.2)	11.8 (5.2–22.8)	.2
CRP (mg/L), mean ± SD	124.8 ± 70.7	40.4 ± 41.4	<.001
Ferritin (ng/mL), mean ± SD	1689 ± 1178	1493 ± 691	.4
D-dimer (ng/mL), mean ± SD	1819 ± 3247	7449 ± 6979	<.001
Fibrinogen (mg/dL), mean ± SD	613 ± 202	398 ± 215	<.001
Prothrombin time (s), mean ± SD	13.9 ± 1.8	16.2 ± 10.7	.2
PC (%), mean ± SD	84.5 ± 16.7	80 ± 23	.4
aPTT (s), mean ± SD	30.2 ± 6.4	37.8 ± 23.3	.2
CK (U/L), mean ± SD	233 ± 254	137 ± 222	.1
CK-MB (U/L), mean ± SD	18 ± 14	20 ± 14	.4
NT-proBNP (pg/mL), mean ± SD	678 ± 752	2282 ± 7754	.3
LDH (U/L), mean ± SD	546 ± 234	663 ± 366	.09
AST (U/L), mean ± SD	66 ± 46	61 ± 40	.6
ALT (U/L), mean ± SD	57 ± 53	76 ± 62	.1

Abbreviations: PT-pulmonary artery thrombosis, HFOT-High-flow Oxygen Therapy, SD-standard deviation, CRP-C-reactive protein, PC-prothrombin concentration, aPTT-activated partial thromboplastin time

Compared to baseline, significant changes were recorded in mean D-dimer values (1819 vs. 7449, p<.001) and CRP (124.8 vs. 40.4, p<.001).

Results - PT developed under therapeutic anticoagulation

Out of nine patients who developed PT under therapeutic doses of LMWHs, in a subgroup of four patients, based on high D-dimer values, a first CTPA was performed, which revealed the absence of PT. In these patients, between the times when both CTPAs were performed, anticoagulation was increased from prophylactic to therapeutic LMWHs doses (Table 5.5).

Table 5.5. Duration of therapeutic LMWH, CTPA and D-dimer changes in patients with a first CTPA excluding PAT

Cases	**Duration of Therapeutic LMWH between CTPAs** (Days)	**D-Dimer (ng/mL)** (When First CTPA Was Performed)	**CTPA Changes** (at PT Diagnosis)	**D-Dimer** (ng/mL) (at PT Diagnosis)
Case 1, 72-year-old man	6	11,000	Main right artery, extended to lobar, segmental and subsegmental arteries	>20,000
Case 2, 69-year-old woman	4	1930	Minor PAT, lower inferior lobe, right lung	9530
Case 3, 76-year-old man	8	14,330	Bilateral PAT—left inferior lobar artery and segmental arteries in middle lobe	2730
Case 4, 81-year-old man	8	5337	Minor PAT, lower inferior lobe, segmental arteries, left lung	8712

Abbreviations: PT-pulmonary artery thrombosis, LMWHs-low-molecular-weight heparins, CTPA-computed tomography pulmonary angiogram

Only one patient, a 59 years-old woman, developed massive PT with hemodynamic instability requiring thrombolysis in the ICU. She had received prior to the diagnosis of PT, since admission, about one week of therapeutic anticoagulation with LMWHs. She had no risk

factors for thrombosis except severe COVID-19. The differential diagnosis was discussed in details and a lot of problems were addressed, especially in a critical COVID-19 case.

Conclusions

In this study, we described clinical, laboratory, and imaging data for 30 patients diagnosed with PT despite receiving prophylactic, intermediate, or therapeutic doses of LMWHs since hospital admission. According to their PADUA score, patients were at increased risk of developing VTE, as no other significant risk factors were recorded for most of them. Most of our patients had a low probability of a diagnosis of PTE according to the Wells score. Anticoagulation regimen, duration, and absence of classic thrombosis risk factors should not be a barrier to exclude this important, life-threatening diagnosis.

Excessive inflammation appears to be the main pathophysiological element, with classical risk factors for thrombosis being mostly absent in these patients. Small, peripheral, segmental/subsegmental thrombi predominate, but cases of massive/high-risk PT are also possible.

Our study also supports current recommendations for therapeutic anticoagulation in patients with severe forms of COVID-19 who are not hospitalized in the ICU.

Literature review

In order to scientifically strengthen the data presented in this study, it was considered useful to review the literature documenting similar clinical cases.

We included data from both cohort studies, case series, and case reports according to the inclusion criteria of confirmed SARS-CoV-2 infection and a diagnosis of developed PT under anticoagulant therapy. We excluded literature review articles and clinical trials in which anticoagulation data were missing or not well specified in relation to the time of the onset of PT.

We analyzed data from 13 cohort studies that included a total of 4058 patients, of whom 346 (8.5%) developed PT, and data from case/series reports, which also included 14 PT patients. Identified PTs were diagnosed in patients with SARS-CoV-2 infection on different anticoagulation regimens, mainly prophylactic with LMWHs, but also intermediate or therapeutic anticoagulation.

From the available data extracted, in the majority of cases, PT was segmental/subsegmental, peripheral, but some patients with COVID-19 also developed imaging significant thrombosis involving the main/lobular pulmonary arteries. High-risk type PT, such as the case presented above, have been described in few patients following review of various published articles.

We also analyzed four cohorts of patients who reported data related to risk factors for thrombosis, prognosis, and biological characteristics (CRP, D-dimers) (Fauvel et al., 2020, Jalde et al., 2021, Niculae et al., 2022, Schiaffino et al., 2021). These included a total of 1445 patients, representing 35% of the total 4058 patients, all of whom were anticoagulated with LMWHs in varying doses. There were 194 (13%) cases of PT diagnosed under anticoagulation therapy.

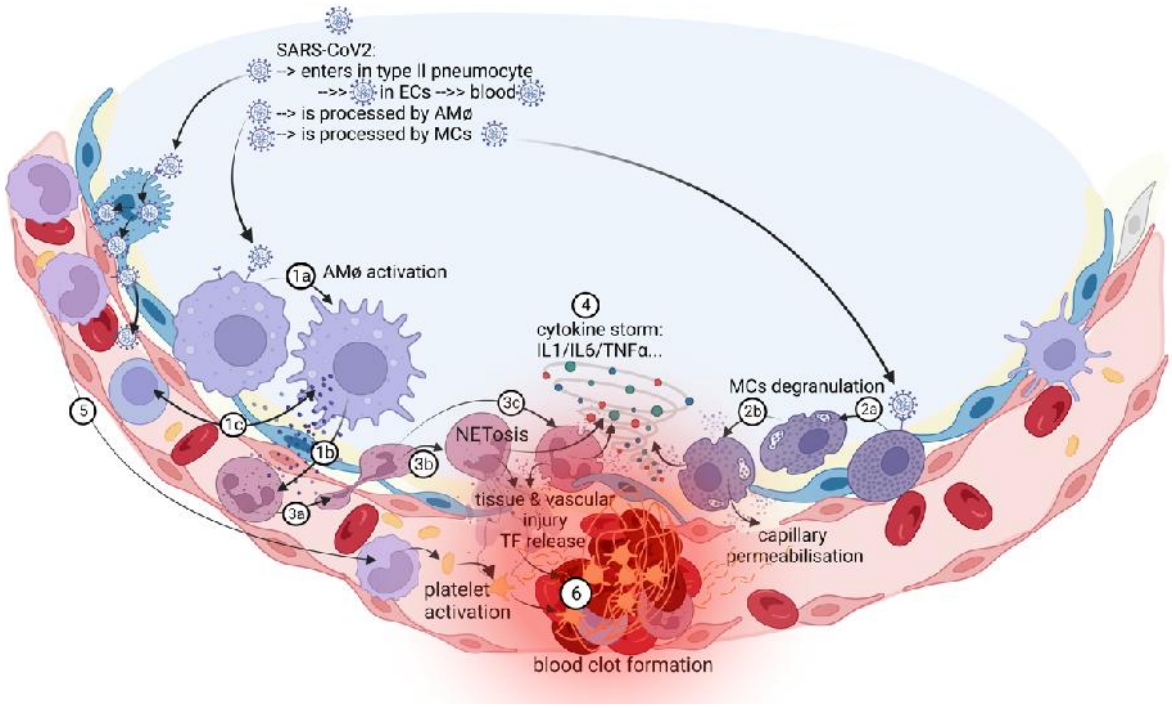
The most important result is that patients with PT did not have statistically significantly more classical risk factors for thrombosis, with the severe form of COVID-19 being more common in the PT group (41.9 vs. 19.3%, $p < .001$) and the condition associated with the development of PT.

Overall in-hospital mortality in the group of patients with PT compared to those without PT was not significantly different (14.4% vs. 12.7%), although the number of those with severe forms of SARS-CoV-2 infection was higher in the PT group.

Objective 1. Biological mechanisms of COVID-19 associated pulmonary artery thrombosis: literature review

In order to provide pathophysiological and immunological support for the data presented above, we also conducted a literature review, relating to pulmonary *in situ* immunothrombosis. We have discussed at length the multiple common pathways of inflammation and thrombosis, with involvement at each biological level.

The results of this theoretical study, some mentioned in the summary of the general part, can be summarized in an original illustration created with a dedicated medical software.



The *in situ* immunothrombosis in COVID-19

Chapter 6. Clinical and imaging features of pulmonary and cardiac involvement in patients with severe COVID-19

In this chapter we have carried out research relating to objectives two and three of this PhD thesis.

Objective 2. To study the impact of treatment with CS and TOC by describing the imaging evolution in patients hospitalized with COVID-19 pneumonia and treated with CS plus TOC and to assess the optimal timing of TOC administration in relation to oxygen flow and correlation with impact on in-hospital mortality.

This objective was achieved in the third original study presented in the PhD thesis, namely "*Impact of Tocilizumab therapy on radiological changes assessed by quantitative chest CT analysis in severe COVID-19* ".

Introduction

In those patients hospitalized with COVID-19 pneumonia, CS and anti-IL-6 immunomodulatory agents such as TOC are essential therapeutic resources to block the "cytokine storm". Data from randomized clinical trials including patients with severe forms of COVID-19 generally support the use of CS, but are inconsistent for TOC, particularly in terms of optimal timing of administration and integration of imaging assessment as a response to therapy.

Materials and methods

The data included in this sub-study were retrospectively analyzed on a group of consecutive adult patients admitted with severe COVID-19 to our department between March 2020 and January 2021.

We included patients who had lung CT evaluation prior to and 7-10 days after TOC administration performed in our clinic. All scans were performed and quantitatively analyzed according to the methodology described above. All patients who had contraindication for TOC and CT evaluation were excluded. We also excluded patients who were administered another immunomodulatory agent and those who had insufficient data for analysis.

Patients were treated with standard of care and dexamethasone, plus at least one dose of TOC, administered at the indication of the clinician, the patient's attending physician, and subject to hospital availability.

Results - Comparative clinical and laboratory data at TOC administration by patient prognosis

Of the 567 patients with SARS-CoV-2 infection admitted, 187 patients had severe forms of the disease. Of these, 101 consecutive patients received TOC and were followed up in this study.

The cohort included 79 (78.2%) male patients with a median age of 61 (51-67) years. Comorbidities were observed in 79 (78.2%) of patients. Oxygen flow had a median of 14 (10-22) L/min, a FiO_2 of 60 (50-60)%, and a PaO2/FiO_2 ratio of 144 (117-194). Prior to TOC administration, the main changes in laboratory parameters resulted in moderate lymphopenia and increased values of inflammation markers and LDH.

All patients had chest CT changes suggestive of consolidation, mixed lesions and interstitial lesions, assessed by volumetric measurement based on HU intervals and expressed as a percentage of total lung volume. All patients had interstitial lesions in at least two lobes and 96% of patients had lesions in all five lobes. Consolidated lesions were present in 36% of patients and were mostly lesions in two lobes in 15.8% of CT scans.

In-hospital mortality in our study was 17.8%, and two groups of patients were created based on this: a first group (survivors) including 83 patients and a second group (deaths) with 18 patients. Comparatively, there was a statistically significant difference in age, presence of comorbidities, Charlson comorbidity index, lymphocyte count, D-dimers, percentage of lung parenchyma affected by interstitial lesions, mixed lesions and alveolar consolidation. Logistic regression found that the percentage of interstitial lesions expressed quantitatively above 50% was associated with death (p=.01). The other variables assessed were age (p=.1), presence of comorbidities (p=.9), oxygen flow rate on TOC administration (p=.2), lymphocyte count (p=.3) and D-dimer level (p=.2).

Results - Comparative analysis of radiological changes between survivors and non-survivors

Comparative analysis of radiological changes between survivors and deceased patients at the time of TOC administration and 7-10 days after are summarised in Table 6.2. In patients who survived, there was a statistically significant improvement in interstitial, alveolar and mixed lesions between the time of TOC administration and 7-10 days after.

Table 6.2. Radiologic changes at 7–10 days after TOC administration, according to the outcome

Radiologic Changes, median (IQR)	**Survivors n = 83**		***p***	**Non-Survivors n = 18**		***p***
	Before TOC	**After TOC**		**Before TOC**	**After TOC**	
Interstitial lesions (%)	39.5 (31.5–48.4)	31.6 (24.7–41.2)	<.001	52 (46.7–60.3)	57.7 (42.7–62.7)	.9
Mixt lesions (%)	4.3 (2.5–7.2)	2.3 (1.5–3.8)	.001	8.2 (4.1–13.7)	6 (3–8.7)	.7
Consolidating lesions (%)	1.7 (1–3.1)	1.1 (0.6–1.6)	.001	3.1 (1.5–7)	2.4 (1.6–4.8)	.2

Abbreviations: IQR = interquartile range, TOC-tocilizumab

Results - Optimal timing of TOC administration

To assess whether the timing of TOC has an impact on in-hospital mortality based on supplemental oxygen requirements at the time of administration, we analyzed specific ROC curves. Thus, the cut-off value for oxygen flow rate was 13 L/min and for FiO_2 was 57.5%, with a significantly higher mortality above these limits (4.7% vs. 27.6%, p=.03). Subsequently, we divided the study population into two groups (Table 6.3).

Table 6.3. Comparative analysis according to the oxygen flow rate and FiO_2 at TOC administration

Variables, Median (IQR)/n (%)	**$FiO_2 \leq 57.5\%$ (Oxygen Flow Rate ≤ 13 L/min) N = 46**	**$FiO_2 > 57.5\%$ (Oxygen Flow Rate > 13 L/min) N = 55**	***p***
Age (years)	57 (50–64)	61 (52–68)	.2
Male sex	37 (80.4)	42 (76.3)	.6
Comorbidities	36 (78.2)	43 (78.1)	.9
Charlson Comorbidities Index	2 (1–2)	2 (1–4)	.09
Symptoms ≤ 11 days	36 (78.2)	46 (79.3)	.6
Symptoms until TOC (days)	9 (7–11)	9 (6.5–11)	.1
Respiratory rate (breaths/minute)	24 (20–30)	27 (22–33.5)	.1
PaO_2/FiO_2 ratio	155 (130–217)	133 (102–173.5)	.02
Lymphocyte count (cells/mm^3)	1053 (700–1500)	808 (537–1000)	.01
Neutrophils/lymphocyte ratio	5.4 (4–11.3)	10 (5.1–15.9)	.2
CRP (mg/L)	45.1 (19.9–86.3)	95.8 (45.5–178)	<.001
D-dimers (ng/mL)	229 (129–367)	261 (156–637)	.1
LDH (U/L)	344 (264.7–409.7)	390 (325–489)	.09
ALT (U/L)	58 (31.5–98.5)	71 (29.2–73.5)	.2
Interstitial lesions (%)	36.1 (27.3–45.1)	47.3 (37.6–55.5)	<.001

Variables, Median (IQR)/n (%)	**$FiO_2 \leq 57.5\%$ (Oxygen Flow Rate ≤ 13 L/min) N = 46**	**$FiO_2 > 57.5\%$ (Oxygen Flow Rate > 13 L/min) N = 55**	***p***
Mixt lesions (%)	3.7 (1.6–4.7)	7.65 (3.8–10.6)	<.001
Consolidation (%)	1.4 (0.8–2.1)	2.9 (1.2–5.7)	<.001
Deaths	2 (4.3)	16 (29.1)	.03

Abbreviations: IQR = interquartile range, TOC = tocilizumab, CRP = C reactive protein, LDH = lactate dehydrogenase, ALT = alanin aminotransferase

Patients requiring oxygen flow rates > 13 L/min (FiO_2 > 57.5%) had lower lymphocyte counts, PaO /FiO_{22} ratio, higher CRP and more severe CT changes..

Conclusions

In our sub-study, 7-10 days after TOC administration, those patients with favorable outcomes showed statistically significant radiological improvement. We also found that interstitial changes of more than 50% were associated with the risk of death in patients with COVID-19 pneumonia.

We proposed the use of oxygen flow rates and FiO_2 as an objective measure that could help in making decisions about the optimal time at which TOC should be administered and calculated the limits at 13 L/min and 57.5%, respectively, with a favorable impact on in-hospital survival.

Objective 3.1 Identify risk factors for the development of pericardial effusion and its impact on patient prognosis

Introduction

Pericarditis is a frequently under-diagnosed complication associated with significantly higher all-cause mortality in patients with SARS-CoV-2 infection (Buckley et al., 2021). Current data regarding pericardial involvement in COVID-19 are limited.

Materials and methods

To achieve the objectives and purpose of this study we performed a retrospective analysis on a group of 100 patients with confirmed SARS-CoV-2 infection hospitalized in our

department from April to December 2020. Patients were divided into two groups: group A (27 patients with pericardial involvement) and group B (73 patients without pericardial involvement).

Lung imaging examinations were performed on a CT scanner according to the methodology described above. We also measured pericardial effusion thickness in the axial, sagittal, and coronal planes (Lazar et al., 2022b).

To establish the diagnosis of pericardial disease, we applied the criteria of the European Society of Cardiology guidelines for the diagnosis and management of pericardial disease (Adler et al., 2015).

Exclusion criteria were lack of CT imaging at admission or sufficient data for statistical analysis in this study and patients with known pericardial involvement.

Results

Of the 567 patients hospitalized in our department, 100 adult patients hospitalized with COVID-19 pneumonia, severe forms of the disease, were included in this sub-study. They were divided into two groups, namely group A (patients with pericardial effusion), with a total of 27 patients, median age 61 (49-68) years, and group B (patients without pericardial effusion), which included a total of 73 patients, median age 61 (51.5-66.5) years.

Pericardial effusion had a median measured thickness of 4 (3-6) mm. We found a small effusion (3-4 mm) in 62.9% of cases and a moderate effusion (5-9 mm) in 37.1% of patients. Only eight patients met criteria for pericarditis, and five of these patients had changes in troponin values in the absence of acute coronary syndrome criteria, along with tachyarrhythmias or atrioventricular block. In these cases we considered the diagnosis of myopericarditis associated with SARS-CoV-2 infection. Patients with pericarditis had a mean fluid effusion size of 7.3 mm, similar to those with myopericarditis (5.2 mm). None of the patients had a clinical or imaging picture compatible with the diagnosis of cardiac tamponade.

In terms of clinical, biological and imaging data, patients with pericardial effusion had higher respiratory rates, more significant changes in coagulation (D-dimers, PAI-1), inflammatory (leukocytes, CRP, ferritin) and cardiac (CK, CK-MB, NT-proBNP and LDH) markers.

We found no statistically significant differences between the two groups of patients in the degree of lung damage on CT imaging. We also identified pleural fluid effusion in 21 cases: 5 (18.5%) in group A (5-22 mm) and 16 (21.9%) in group B (3-65 mm).

Mortality for the whole group studied was 24% (24 patients), with a higher percentage in group A (33.3%) compared to group B (20.8%).

The diagnosis of pericardial effusion had the best statistical correlation with myoglobin and CK values. Complementary to the logistic regression assessment, the Spearman correlation test additionally showed the same for some markers of inflammation, namely CRP levels (Table 6.6).

Table 6.6. Correlations of clinical and laboratory parameters with pericardial effusion

Laboratory data	Spearman's Rho	*p*
CRP	0.201	.05
Platelets	0.198	.05
Myoglobin	0.408	<.001
CK	0.325	.001
CK-MB	0.242	.01
LDH	0.261	.009

In terms of clinical prognosis beyond mortality rates, patients with SARS-CoV-2 infection and associated pericardial effusion had higher rates of ICU admission (14.8% vs. 4.1%) at the limit of statistical significance (p = .06).

Conclusions

Pericardial effusion had a high prevalence of 27% in our cohort of patients with severe forms of COVID-19, although in most cases its size was small, with only 30% of cases developing in the context of clinical criteria for pericarditis.

Increased values of cardiac enzymes (myoglobin, CK, CK-MB), LDH, platelets and CRP were associated with the presence of pericardial effusion, with myoglobin having the highest statistical correlation index. We did not identify comorbidities that particularly predisposed to the occurrence of this cardiac condition, nor did we identify an association with the degree of

pulmonary impairment, but the mortality of these patients was higher compared to the control group.

Objective 3.2. The analyze of mortality risk factors in SARS-CoV-2 infection with integration of quantitative CT imaging into a prognostic score

Introduction

Current models applicable to SARs-CoV-2 infection in terms of progression and prognostic scores used in the evaluation and management of severe forms of the disease have included only qualitative or semi-quantitative lung imaging assessments of associated parenchymal inflammatory lesions (Charles et al., 2008, Guo et al., 2019), which may lead to less conclusive results. .

Materials and methods

To achieve the objectives and purpose of this study we conducted a retrospective analysis on a group of 100 patients with confirmed SARS-CoV-2 infection hospitalized in our department from April to December 2020.

Lung imaging examinations were performed and quantitatively analyzed according to the general methodology described. In addition, we further analyzed normal lung densities using a *cluster* analysis method that calculates and displays volumes of connected (3D) voxels in a specified density range. For normal lung densities, we used the following cluster types: cluster 1 (C1) - between 2 and 10 mm^3 , cluster 2 (C2) - between 11 and 60 mm^3 , cluster 3 (C3) - between 61 and 200 mm^3 and cluster 4 (C4), above 201 mm^3 (Lazar et al., 2022a).

Also, in this subchapter we have detailed the statistical methodology behind the development of *the COV score*, which has been compared with those existing in the literature (*MuLBSTA, SMART-COP*) in order to estimate COVID-19 pneumonia-associated mortality.

Results - Clinical, biological and imaging data

Of the 567 patients hospitalized in our department, 100 adult patients hospitalized with COVID-19 pneumonia, severe forms of the disease, were included in this sub-study. They were divided into two groups, namely group A (survivors) with a total of 76 patients, median age 57.5 (49.2-65) years and group B (deceased patients) with 24 patients, median age 66 (62-71) years. We found no statistically significant differences in the type of comorbidities. All patients

included in the study had severe clinical forms of SARS-CoV-2 infection, with higher flow rates for group B patients (median 25 L/min vs. 12, p = .003) and more frequent MV required (12.5% vs. 0, p = .003).

Also, in group B patients, lower values were detected for PaO_2/FiO_2 ratio, absolute lymphocyte count, platelet count, hemoglobin and higher values for neutrophils, BNP, D-dimers, CK, LDH and serum creatinine compared to group A, without quantifying statistically significantly different values for other parameters.

Imaging characterization of lung parenchymal lesions using semi-quantitative tools (number of lung lobes with pathological changes) was not significantly different between the two groups of patients (Table 6.11).

Table 6.11. CT lung involvement in survivors (group A) and non-survivors (group B) at hospital admission

Parameter	Group A Median [Q1, Q3]	Group B Median [Q1, Q3]	*p*-Value
Alveolar lesions (%)	1.8 [1; 3.2]	3.1 [1.5; 5.1]	.06
Mixt lesions (%)	4.6 [2.5; 7.3]	6.9 [4.1; 12]	.03
Interstitial lesions (%)	39.4 [31.7; 47.8]	49.2 [44.3; 60.1]	.001
Total lung involvement (%)	47.2 [35.9; 63]	64.9 [48.1; 74.1]	.003
Normal lung densities (%)	52.7 [37; 64.1]	35.1 [25.9;51.9]	.003
Cluster 1 (2–10 mm^3) (%)	0.4 [0.2; 0.9]	1.1 [0.3; 1.5]	.02
Cluster 2 (10–60 mm^3) (%)	0.45 [0.2; 0.9]	0.9 [0.3; 1.7]	.01
Cluster 3 (60–200 mm^3) (%)	0.1 [0; 0.3]	0.4 [0.2; 0.6]	.001
Cluster 4 (over 200 mm^3) (%)	51.6 [35.5; 63.7]	33 [20.7; 51.7]	.002
Lobes with interstitial lesions (*n*)	5 [5; 5]	5 [5; 5]	.9
Lobes with alveolar lesions (*n*)	0 [1; 2]	0 [0; 2]	.3
Lobes with atelectatic changes (*n*)	3.5 [2; 5]	2 [2; 4]	.2

Multivariate logistic regression performed using independent variables of quantitative imaging parameters (percentage alveolar, mixed, interstitial lesions, total lung damage) showed statistical significance (p = .008).

Results - Mortality risk factors

A lower percentage of normal lung densities, PaO_2/FiO_2 ratio, lymphocyte, platelets, haemoglobin and serum albumin ratios, along with a higher percentage of interstitial lung injury, oxygen flow, FiO_2, neutrophil/lymphocyte ratio, LDH, CK-MB, myoglobin and serum creatinine are associated with significantly higher mortality (Table 6.12).

Table 6.12. Risk factors associated with mortality

Parameter	***Pearson* Correlation** *(Point Biserial)*	***p***	**OR (95% CI)**	**Change in Mortality (%)**
Age (years)	0.260	.009	1.052 (1.011; 1.094)	5.2 *
O_2 flow (L/min)	0.306	.002	1.092 (1.029; 1.159)	9.2 *
FiO_2 (%)	0.260	.009	1.045 (1.009; 1.083)	4.5 *
PaO_2/FiO_2 ratio	−0.235	.02	0.99 (0.982; 0.999)	1 #
Lymphocytes ($\times 10^3/\mu L$)	−0.360	<.001	0.997 (0.995; 0.999)	0.3 #
Neutrophils/lymphocytes	0.341	.001	1.067 (1.023; 1.113)	6.7 *
Platelets ($\times 10^3/\mu L$)	−0.302	.002	0.99 (0.984; 0.997)	1 #
Hemoglobin (g/dL)	−0.362	<.001	0.623 (0.47; 0.83)	38 #
CKMB (U/L)	0.258	.01	1.047 (1.004; 1.091)	4.7 *
LDH (U/L)	0.371	<.001	1.005 (1.002; 1.008)	0.5 *
Serum albumin (g/L)	−0.450	<.001	0.062 (0.012; 0.32)	93.8 #
Myoglobin (µg/L)	0.282	.013	1.006 (1.001; 1.011)	0.6 *
Interstitial lesions (%)	0.320	.001	1.065 (1.022; 1.109)	6.5 *
Total lung involvement (%)	0.335	.001	1.049 (1.018; 12.08)	4.9 *
Normal lung densities (%)	−0.335	.001	0.953 (0.926; 0.982)	4.7 #
Cluster1 (2–10 mm^3) (%)	0.209	.04	1.798 (1.015; 3.183)	79.8 *

Parameter	*Pearson* Correlation *(Point Biserial)*	*p*	OR (95% CI)	Change in Mortality (%)
Cluster2 (10–60 mm^3) (%)	0.233	.01	1.936 (1.081; 3.467)	93.6 *
Cluster 3 (60–200 mm^3) (%)	0.241	.01	4.92 (1.262; 19.181)	392 *
Cluster 4 (> 200 mm^3) (%)	−0.341	.001	0.956 (0.93; 0.98)	4.4 #

* for 1 unit increase in the parameter; # for 1 unit decrease in the parameter. OR = odds ratio; PaO_2 = arterial O_2 pressure, FiO_2 = fractional inspired oxygen, CKMB = creatine kinase MB, LDH = lactate dehydrogenase

Results - Regression model for mortality

The impact of demographic, haematological, biological, radiological and respiratory characteristics on survival, mortality rates and overall model accuracy were assessed by logistic regression. Among all parameters analysed by binary logistic regression, Pearson correlation and multivariate logistic regression, we selected the following parameters to establish an optimal regression model: age, lymphocytes, PaO_2/FiO_2 ratio, percentage of total lung injury, LDH, serum albumin, D-dimers, oxygen flow rates and myoglobin (Table 6.13).

Table 6.13. Regression model to evaluate mortality rate in patients with severe COVID-19 pneumonia

	Omnibus Test of Model Coefficients	Nagelkere R^2	*Hosmer Lemeshow* Test	Corrected Survival Rate (%)	Corrected Mortality Rate (%)	Overall Accuracy Prediction (%)
Regression model	<.001	0.699	0.877	94.3 (97.1*)	78.6 (85.7*)	89.8 (93.9*)

* increase for the corrected survival rate to 97.1%, for the corrected mortality rate to 85.7% and for the overall accuracy prediction to 93.9% if we substitute the parameter "lung involvement" from the regression model with the parameters presented in the cluster analysis (Cluster 1 to Cluster 4)

Results - Development of a prognostic score (COV)

An ROC curve analysis was performed for each of the variables in the regression model to identify the *cut-off* value with optimal sensitivity and specificity: age, PaO_2/FiO_2 ratio, percentage of lung injury, LDH, serum albumin, D-dimers, oxygen flow rates and myoglobin.

The data obtained were used to create a prognostic score for patients with severe forms of COVID-19 (*COV score*). We assigned 2 points for percentage of lung damage > 60%, and 1 point for each of the following: age > 65 years, lymphocytes < 775 ($\times 10^3$ /μL), PaO_2/FiO_2 ratio < 140, LDH > 450 U/L, serum albumin < 3.6 g/L, D-dimers > 290 ng/mL, oxygen flow rates > 14.5 L/min and myoglobin > 235 μg/L. Points were awarded based on OR of the parameters in the regression model (1 point for each parameter with OR < 1.1). Considering the significant impact of radiological parameters in characterizing mortality (OR ranging from 0.95 to 4.92), we allocated 1 additional point for the variable *total lung injury*.

We compared *the COV score* obtained using logistic regression with similar scores that included radiological quantification of lung injury as a variable (MuLBSTA and SMART-COP), obtaining better results in terms of mortality prediction (85.9% vs. 78.8% vs. 79.8%).

Both the calculation formula for the score and the associated probability of death were also described. Table 6.16 shows the probabilities of death for all VOC score values from 1 to 10 points.

Table 6.16. Probability of death for COV-Score

COV score value	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Probability of death (%)	0	1.5	3.3	7.3	15	28.4	47	66.6	81.7	90.1	95.7
Observed frequency of death (%)	0	0	0	9.1	11.1	30	40	80	100	100	100

In addition, we performed an ROC curve analysis to verify the logistic regression results and prediction accuracy for each development score.

The COV score had a larger area under the ROC curve (AUC = 0.884, $p < .001$) compared to MuLBSTA and Smart-COP, and was better in predicting mortality for this subgroup of patients with severe forms of COVID-19.

Conclusions

Quantitative imaging assessment of lung inflammatory lesions improves prediction algorithms compared to semi-quantitative parameters, and therefore the data presented highlight the importance of this examination to be used as a standard radiological parameter in terms of COVID-19 pneumonia assessment. Serum albumin, LDH value, lymphocyte count and

percentage of lung parenchymal injury were the markers best associated with an increased risk of mortality in our study.

The proposed COV score is a good alternative to the scores currently used in the literature (MuLBSTA and SMART-COP), demonstrating both better sensitivity and specificity in predicting patient prognosis at the time of hospital admission.

Final conclusions

SARS-CoV-2 infection has been a real challenge for the medical world. The complex immunopathological considerations related to inflammation and pulmonary thrombosis pose major evaluation and treatment issues, which we have addressed within the scope of this scientific paper.

The results of our personal research have capitalized on quantitative lung CT imaging, which allowed us to assess the association between the occurrence of pulmonary thrombotic events and inflammation in the adjacent lung parenchyma. Moreover, these results also suggest the need for a more intense anticoagulation regimen, and a combination of anti-inflammatory, immunomodulatory and anticoagulation therapy to prevent pulmonary thrombotic events. These considerations have been further validated in international guidelines, which have reached a consensus for therapeutic anticoagulation in patients with severe forms of the disease outside the ICU.

Also, regarding immunomodulatory therapy, we have proven its usefulness in this pathology in terms of patient prognosis without significant adverse reactions in the study. Thus, we integrated quantitative lung CT imaging data in an optimal way to quantify the impact of anti-IL-6 immunomodulatory therapy with TOC and CS on the clinical-imaging evolution of patients with severe forms of COVID-19. Also, given the lack of data in the literature regarding the optimal timing of TOC administration, we performed a statistical analysis that allowed us to determine this by relating oxygen flows and FiO_2, with prognostic impact. The accumulated data related to OCD confirmed its usefulness and indication in severe forms of COVID-19, even becoming an indication in many international guidelines and local protocols.

Since the course of this acute infectious disease is closely linked to the existence of mortality risk factors, important data in this paper focused on identifying these. In the last part we developed a new prognostic score in SARS-CoV-2 infection, the "COV" score, which

integrates for the first time in the literature quantitative CT imaging data in quantifying patient prognosis. Numerous clinical and biological data, in addition to imaging data, were considered and we highlighted the role of this score in the early identification, as early as hospital admission, of patients at significant risk of adverse outcome. Not only the pulmonary impairment assessed imaging, but also the cardiac assessment by CT examination was addressed in terms of risk factors that may contribute to the occurrence of pericardial effusion and its impact on the prognosis of patients more likely to be admitted to the ICU and with clinical significance in terms of associated mortality.

The major novelty worth mentioning in an overall framework of this clinical research is the integration of imaging aspects in the assessment of patients with COVID-19 pneumonia, some described for the first time in the literature. A novel pathophysiological mechanism of pulmonary *in situ* immunothrombosis was supported by clinical and imaging data, correlated with a novel methodology targeting anticoagulation failure, in an extensive literature review framework in which we integrated numerous immunopathological details in order to support these aspects. I also consider that the paper has achieved its objectives that have outlined the purpose related to the evaluation and treatment of these patients and also opens new research directions, as evidenced within each subchapter.

To the best of the authors' knowledge, to date, this PhD thesis is among the first developed at the University of Medicine and Pharmacy "Carol Davila" and in the country to provide clinical and imaging data on SARS-CoV-2 infection. Numerous recent references were used, especially in the general part, mostly indexed in *Web of Science*, with an impact factor. From the PhD studies, 7 scientific articles were published. Of these, 5 articles were of original type and 2 of literature review type. The cumulative impact factor for the 5 articles as main author (which are also the validated ones) is 18.8. In terms of scientific visibility of the doctoral research, so far, the 7 articles have in total 27 citations in *Web of Science*.

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2. Niculae, Cristian-Mihail; Hristea, Adriana; Albulescu, Andreea Simona, Petre, Vladimir Bogdan; Anghel, Ana-Maria-Jennifer; Damalan, Anca-Cristina; Bel, Adela-Abigaela; Lazar, Mihai. *Quantitative chest CT imaging characteristics and outcome of patients with COVID-19 associated pulmonary artery thrombosis: A single-center retrospective cohort study.* Medicine 102(27):p e34250, 2023**.** PMID: 37417640; PMCID: PMC10328685. **WOS:001025647200026**. **Impact factor: 1.6** (2023); Original Article. DOI: 10.1097/MD.0000000000034250.
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B. Co-author

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