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Pulmonary thrombosis associated with COVID-19 pneumonia: Beyond classical pulmonary thromboembolism

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Abstract

Background: Classical pulmonary thromboembolism (TE) and local pulmonary thrombosis (PT) have been suggested as mechanisms of thrombosis in COVID-19. However, robust evidence is still lacking because this was mainly based on retrospective studies, in which patients were included when TE was suspected.

Methods: All patients with COVID-19 pneumonia underwent computed tomography and pulmonary angiography in a prospective study. The main objective was to determine the number and percentage of thrombi surrounded by lung opacification (TSO) in each patient, as well as their relationship with percentage of lung involvement (TLI), to distinguish classical TE (with a random location of thrombi that should correspond to a percentage of TSO equivalent to the TLI) from PT. We determined TLI by artificial intelligence. Analyses at patient level (TLI and percentage of TSO) and at thrombi level (TLI and TSO) were performed.

Results: We diagnosed TE in 70 out of 184 patients. Three (2–8) thrombi/patient were detected. The percentage of TSO was 100% (75–100) per patient, and TLI was 19.9% (4.6–35.2). Sixty-five patients (92.9%) were above the random scenario with higher percentage of TSO than TLI. Most thrombi were TSO ($n = 299$, 75.1%). When evaluating by TLI (<10%, 10%–20%, 20%–30% and >30%), percentage of TSO was higher in most groups. Thrombi were mainly in subsegmental/segmental arteries, and percentage of TSO was higher in all locations.

Conclusions: Thrombi in COVID-19 were found within lung opacities in a higher percentage than lung involvement, regardless of TLI and clot location, supporting the hypothesis of local PT rather than "classic TE".

KEYWORDS

COVID-19, pneumonia, prospective, pulmonary embolism, Pumonary thrombosis, SARS-CoV-2

1 | INTRODUCTION

COVID-19 has heterogeneous clinical features, from asymptomatic presentation to acute respiratory distress syndrome, with significant mortality rates.[1] Moreover, thrombotic complications have been described during the disease,[2,3] being pulmonary embolism (TE) the most frequent one,[4–8] with prevalence rates ranging from 7%–50%[9,10] and substantial variability by COVID-19 severity as well as certain underlying medical comorbidities. Besides, PE has been associated with worse prognosis.[11]

Despite its high incidence and clinical implications, the etiopathogenesis of TE in COVID-19 remains unclear. Some complex and interconnected mechanisms, including haemostasis, vascular integrity and inflammation, have been suggested.[12] COVID-19 is associated with hypoxia and severe inflammatory status, which, among others, could lead to endothelial damage and thrombocytopathy, all of which induce a hypercoagulable state.[12] It has been hypothesised that pulmonary thrombosis (PT) could be an "in situ" phenomena rather than a classical TE.[13] Some of the reasons are that thrombi are quite frequent despite the use of thromboprophylaxis; moreover, they have been found much more frequently in the lungs than in any other organ[14] with a predominant segmental distribution,[5,6] as it is the case with the location of consolidation areas. Besides, deep vein thrombosis (DVT) has not been identified as frequently as TE.[3,15] In addition, the increased inflammatory and prothrombotic status may be even more locally amplified in COVID-19 pneumonia, as revealed by postmortem studies. These showed multiple and small pulmonary thrombi, commonly in areas with diffuse alveolar damage, as well as a local increase in inflammatory, endothelial dysfunction mediators[16,17] and immunothrombosis.[18] Furthermore, some studies with computed tomography pulmonary angiography (CTPA) showed a greater degree of lung involvement in patients with TE.[13,19] It is interesting to note that some studies reported that the thrombi were predominantly located in areas with parenchymal abnormalities,[6,13,20,21] although other studies did not.[5,6] Nevertheless, pathological studies showed data only from fatal COVID-19, and most clinical studies were limited mainly due to their retrospective design and the inclusion of patients who underwent CTPA only when TE was suspected. Besides, neither the location of thrombi within lung opacification nor the adjustment for the percentage of lung parenchymal involvement were evaluated.

Overall, all of the above seems to indicate that severe COVID-19 is associated with a strong, sustained inflammatory response and a prothrombotic status that could lead to local PT.

2 | METHODS

2.1 | Study design and participants

The main objective was to determine the number and percentage of thrombi surrounded by lung opacification (TSO) in each patient with COVID-19 pneumonia, as well as their relationship with percentage of lung involvement (TLI), to distinguish classical TE from local thrombosis. This was determined in a prospective study using CTPA in every consecutive patient admitted to the hospital and with at least one D-dimer measurement >1000 ng/mL, regardless of clinical suspicion.[5] As secondary objectives, we aimed to: (1) analyse the percentage of TSO in the total number of thrombi (NT), according to their location, and TLI; (2) compare anthropometric and clinical characteristics of patients, baseline laboratory data, inflammatory profile and pulmonary embolism biomarkers according to the percentage of TSO in each patient.

We selected all patients with TE from a previous prospective study to evaluate the incidence of TE in COVID-19.[5] Briefly, all consecutive patients with COVID-19 pneumonia who were admitted to the hospital and had at least one D-dimer measurement >1000 ng/mL, regardless of clinical suspicion of TE, underwent a CTPA.

The diagnosis of COVID-19 pneumonia was done according to the case definition established by WHO interim guidance.[22] Patients were excluded if they were on anticoagulant treatment in the 3 months before admission or if they were unable to undergo a CTPA for any of the following reasons: unwillingness or inability to participate in the study, allergy to iodinated contrast, or any other concurrent clinical condition that would contraindicate their participation in the study.

2.2 | Description of undertaken investigations

Epidemiological, demographic, laboratory examinations, medical treatment, respiratory support, clinical outcomes, TE risk factors and Pulmonary Embolism Severity Index (PESI)[23] were collected in all patients.

Laboratory data included complete blood count, coagulation and kidney and liver function tests taken upon admission. In addition, baseline and peak of the following biomarkers were analysed in each patient: fibrinogen, D-dimer, erythrocyte sedimentation rate (ESR), interleukin-6 (IL-6), interleukin-10 (IL-10), ferritin, C-reactive protein (CRP) and lactate dehydrogenase (LDH). High-sensitive troponin I, N-terminal prohormone B-type natriuretic peptide (NT pro-BNP) and blood gas parameters were also measured. More details are shown in Data S1.

2.3 | Computed tomography pulmonary angiography

TLI (lung with ground-glass opacities, crazy-paving and/or consolidations) was automatically calculated by artificial intelligence analysis [InferReadTM CT Lung (COVID-19); Infervision, Europe GmbH, Germany].[24] Right ventricle to left ventricle diameter ratio (RV/LV) was measured.[25] For each patient, two radiologists evaluated the CTPA scans for the following characteristics: (a) direct visualisation of the endoluminal thrombi, (b) NT and location of each thrombus, (b) presence of lung opacification surrounding each pulmonary artery thrombus (TSO), being defined as ground-glass opacities or consolidation in lung parenchyma 10 mm away or less from each pulmonary vessel with the thrombus (Figure 1).

2.4 | Statistical analysis

Descriptive statistics included frequencies and percentages for categorical variables and medians and interquartile ranges (IQRs) for continuous variables. Comparisons between each patient's numerical variables were determined by the Wilcoxon paired samples test, while the Kruskal–Wallis test or Mann–Whitney *U* test were used for comparisons between groups.

Two datasets were analysed in parallel: (1) aggregated pulmonary thrombi data at patient level, (2) disaggregated data at pulmonary thrombi level. The following numerical variables were considered in the first dataset: (a) percentage of total lung involvement (TLI), (b) total number of thrombi (NT) and (c) percentage of thrombi surrounded by lung opacification; all dimensions were

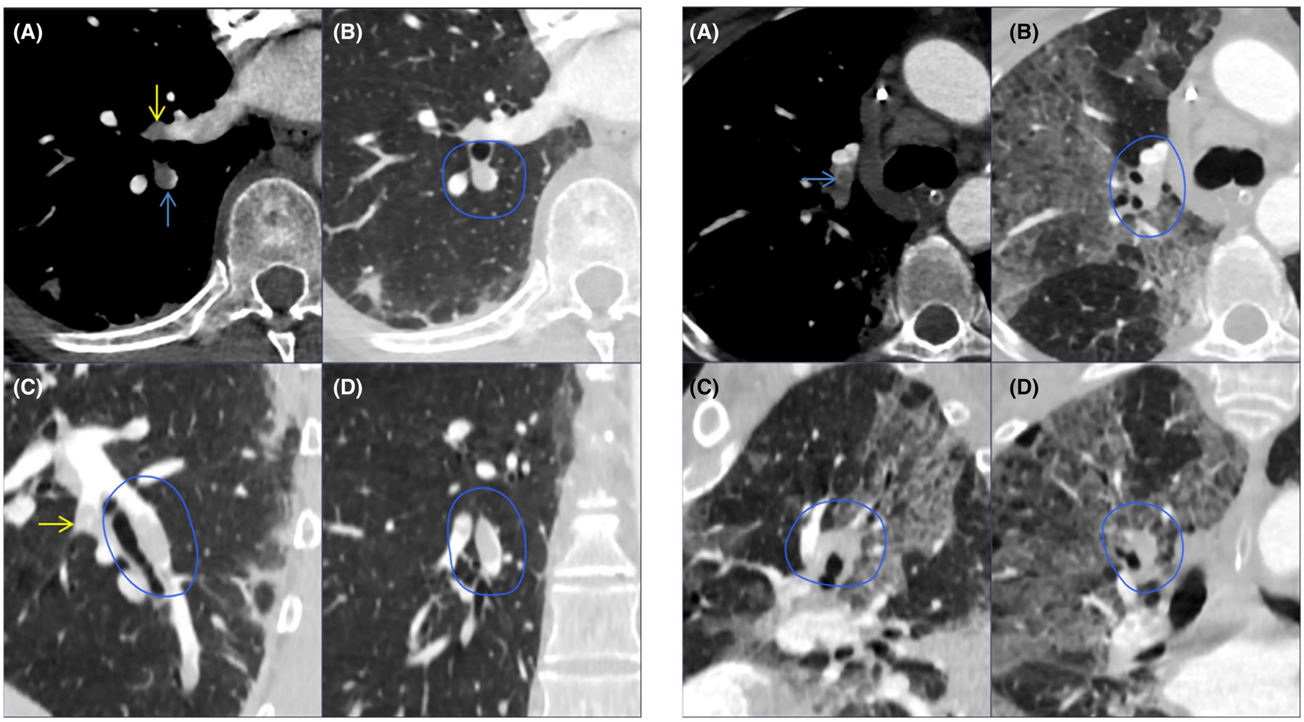

FIGURE 1 Calculation of lung opacifications surrounding pulmonary artery thrombosis. Right panel: lung opacification surrounding pulmonary artery thrombus (blue arrow). CTPA, thin section axial (A, B), sagittal (C) and coronal (D) show moderate–severe lung opacification less than 10 mm from the pulmonary vessel with the thrombus (the blue area depicts the parenchyma of interest). Left panel: pulmonary thrombus (blue arrow) without surrounding lung opacification. CTPA, thin section axial (A, B), sagittal (C) and coronal (D) showed presence of peripheral lung opacification but 10 mm away or more from the pulmonary vessel with the thrombi (the blue area depicts the lung parenchyma of interest). There were also other thrombi (yellow arrow) that required individualized assessment.

measured for each patient. An analysis was carried out using scatter graphs to visualise the relationship between the TLI and the percentage of TSO, and they were disaggregated by NT groups. It was presumed that a random location of pulmonary thrombi should correspond to a percentage of TSO equivalent to the TLI in patients with classical PE. The random scenario was graphed in the figure with the perfect fit line. To contrast the non-concordance observed between the percentage of TSO and the TLI, we used the Wilcoxon paired sample method. On the other hand, the variables considered in the second dataset were: (a) TLI and (b) thrombi surrounded by lung opacification (TSO) as a dichotomous variable. Percentage of TSO was calculated and assessed by the binomial contrast test between two categories with equal probability (50%). Subsequently, percentage of TSO were also disaggregated by TLI levels (<10%, 10%–20%, 20%–30% and >30%) to adjust the prevalence rate. TLI differences between TSO versus those placed in non-infiltration lung locations were evaluated using *U*-Mann Whitney test. Differences were considered statistically significant at 2-tailed $p<.05$. Data analysis was carried out by the Methodological and Statistical Support Platform of IdISBa using the statistical software SPSS v.26 (IBM Corporation, USA).

3 | RESULTS

3.1 | COVID-19 pneumonia population

A total of 1798 COVID-19 patients were hospitalised during the study period (April 6, 2020–February 2, 2021). Overall, 324 of those patients had pneumonia with D-dimer >1000 ng/mL, and 142 of them presented at least one exclusion criterion. CTPA was performed on 182 patients, three of which were excluded due to invalid CTPA (Figure 2). A total of 71 patients had TE, one of which was excluded because of inappropriateness for the subsequent CTPA measurements.

3.2 | Pulmonary artery thrombi analysis at patient level

A total of 70 TE patients were included in the analysis. Table 1 contains baseline anthropometric and clinical characteristics of the TE patients included in this study. TLI was 19.9% (4.6–35.2) in all patients. TE patients had a median of three thrombi (2–8), which were 100% (75%–100%) TSO.

Figure 3 shows the scatter graph analysis of the relationship between the TLI and the percentage of TSO disaggregated by NT in each patient. A total of 65 patients (92.9%) were above the random scenario that was graphed with the perfect fit line, and they had higher percentage of TSO than TLI, which was significantly different than in the 5 (7.1%) patients who had higher TLI than percentage of TSO ($p<.001$). Furthermore, 41 patients (58.6%) had all their thrombi surrounded by pulmonary opacification regardless of TLI (Figure 3). The group of 5 patients who had higher TLI than percentage of TSO were older (77 (66–83) vs. 63 (55–72) years, $p=.045$) and had a lower peak D-dimer value (1892 (1080–3007) vs. 3489 (2575–8585) ng/mL, $p=.01$). In addition, TLI was significantly higher in patients who had all their thrombi surrounded by lung opacification compared with those patients with some or none of their thrombi surrounded by lung opacification (Figure S1). However, the disaggregated analysis by NT showed similar results since there were no differences in TLI and percentage of TSO among all groups, and most patients (around 100%) had higher percentage of TSO than TLI ($p<.05$; Figures S2 and S3).

3.3 | Comparison of clinical characteristics, RV dysfunction and laboratory findings between patients according to percentage of TSO

Age, sex, anthropometric, clinical characteristics, physical examination, TE risk factors, PESI and treatment during hospitalisation were not significantly different between patients with no TSO ($n=5$), patients with some TSO ($n=24$) and patients with all TSO ($n=41$). Moreover, no differences were found in clinical outcomes or in RV/LV according to the percentage of TSO (Figure S4).

Baseline laboratory findings, as well as inflammatory and TE biomarkers are shown in Tables 2 and 3. Patients with no TSO showed a lower total cholesterol value when compared to patients who had some or all TSO. There were no significant differences in baseline coagulation function or arterial blood gas tests. In addition, significantly lower peak CRP and D-dimer levels, higher baseline red blood cells distribution width (RDW) and peak platelet lymphocyte ratio (PLR) values were found in patients with no TSO. Moreover, peak platelet distribution width (PDW) and IL-10 concentration were found to be higher in patients with all TSO when compared to patients with some TSO, while no differences were detected in LDH, ESR, ferritin, platelet count, lymphocyte count, neutrophil-to-lymphocyte ratio (NLR), IL-6, NT-proBNP, troponin, or fibrinogen values (Table 3).

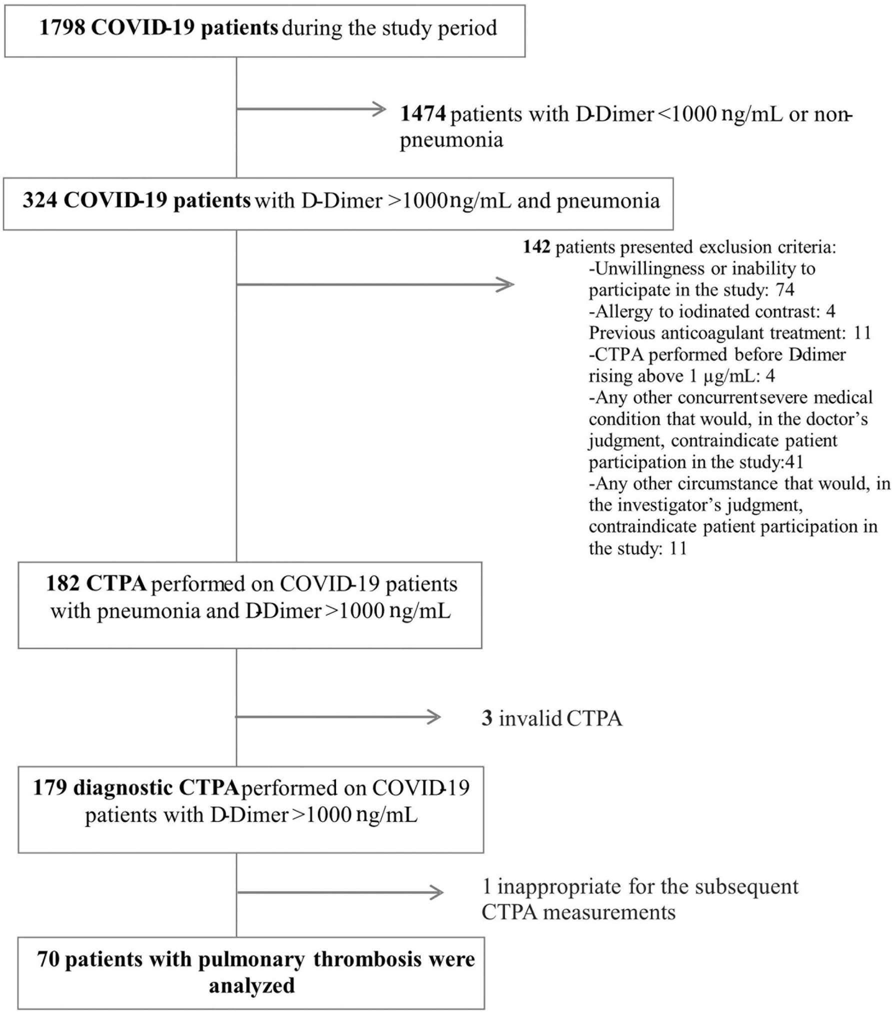

FIGURE 2 Flow chart.

3.4 | Pulmonary artery thrombi analysis at thrombi level

Among the 70 TE patients, a total of 398 thrombi were evaluated, and most of them (n=299, 75.1%) were TSO (p<.001). TLI was higher in TSO compared with pulmonary artery thrombi not surrounded by lung opacification (20.5% vs. 2.2%, respectively, p<.001; Figure S5). Consequently, we performed an analysis of percentage of TSO disaggregated by TLI to adjust the prevalence rate due to lung infiltration (Figure 4). There were no differences in TLI between TSO and those thrombi not surrounded by lung opacification in most groups. TLI was only slightly higher in TSO within the group of thrombi located in lungs with TLI <10% (3.4% vs. 1%, p<.001). Furthermore, there were more than 50% of TSO, which was much higher than the distribution of thrombi with respect to a random percentage reference to a distribution equal to the TLI in which they were located. Finally, we found a higher significant percentage of TSO than percentage on non-TSO in most of the TLI groups (<10%, 10–20%, 20–30% and >30%).

Among the 398 thrombi, they were located mainly in subsegmental (n=178) and segmental (n=188) pulmonary arteries, and only 33 thrombi were found in main/lobar arteries. Percentage of TSO was significantly higher in all locations (subsegmental=80%; segmental=68%; main/lobar=91%, p=.004).

4 | DISCUSSION

The null hypothesis presumed that a random location of pulmonary thrombi in COVID-19 pneumonia should correspond to a percentage of TSO in each patient equivalent to the TLI. However, we found a high overlap between thrombi location and consolidation areas, and most of the thrombi were TSO, regardless of TLI, and unrelated of thrombus location. This supports the alternative hypothesis that immunothrombosis phenomena could lead to in situ pulmonary artery thrombi formation.

There are also some other clinical considerations to support this hypothesis. First, most studies found that

TABLE 1 Baseline anthropometric and clinical characteristics of patients.

	All patients ($n=70$)
Age, yrs.	65 (56–73)
Sex, male, *n* (%)	51 (72.9)
Body mass index, Kg/m^2	26.6 (26.6–31.6)
Cardiovascular disease, *n* (%)	16 (22.9)
Arrhythmia, *n* (%)	2 (2.9)
Chronic respiratory disease, *n* (%)	7 (10)
Previous antiplatelet treatment, *n* (%)	9 (12.9)
Time from symptom onset to hospital admission, days	7 (4–11)
Current or former smokers, *n* (%)	26 (37.2)
Smoking, Pack-year	0 (0–10)
D-dimer, ng/mL	1013 (324–4474)
Symptoms	
Cough, *n* (%)	53 (76.8)
Fever, *n* (%)	52 (74.3)
Dyspnea, *n* (%)	41 (58.6)
Hemoptysis, *n* (%)	1 (1.4)
Chest pain, *n* (%)	6 (8.6)
Physical examination	
Respiratory rate, breaths per min	22 (20–26)
Heart rate, beats per min	86 (74–104)
Systolic BP, mm Hg	126 (117–135)
Diastolic BP, mm Hg	71 (63–80)
Temperature, °C	37 (36.1–37.6)
Lower limb edema, *n* (%)	2 (2.9)
PESI	95 (76–111)
Strong-moderate PE risk factors	
Heart failure, *n* (%)	3 (4.3)
Chronic respiratory failure, *n* (%)	1 (1.4)
Neoplasm, *n* (%)	4 (5.7)
Previous VTE, *n* (%)	1 (1.4)
Myocardial infarction (within previous 3 months), *n* (%)	1 (1.4)
One or more known risk factors for PE, *n* (%)	10 (14.3)
Treatment in hospital	
Oxygen therapy	
Maximum FiO_2	1 (.4–1)
HFNC, *n* (%)	19 (27.1)
NIV, *n* (%)	2 (2.9)
IMV, *n* (%)	24 (34.3)
Pharmacological therapy	
Azithromycin, *n* (%)	10 (14.3)
Hydroxychloroquine, *n* (%)	18 (25.7)
Remdesivir, *n* (%)	9 (12.9)
Tocilizumab, *n* (%)	19 (27.1)
Other biological therapy, *n* (%)	4 (5.7)
Systemic steroids, *n* (%)	63 (90)
Clinical outcomes	
Acute respiratory failure, *n* (%)	52 (76.5)
Arrhythmia, *n* (%)	2 (2.9)
ICU admission, *n* (%)	33 (74.1)
Death, *n* (%)	6 (8.6)

Note: Values represent percentage or median (IQR) according to its distribution.

Abbreviations: BP, blood pressure; CTPA, computed tomography pulmonary angiography; FiO2, fractional inspired oxygen; HFNC, high flow nasal cannula; ICU, intensive care unit; IMV, invasive mechanical ventilation; IVF, in vitro fertilization; NIV, non-invasive ventilation; PE, pulmonary embolism; VTE, Venous thromboembolism.

thrombi were mostly peripheral (segmental and subsegmental), overlapping with COVID-19 usual parenchymal lung consolidations.[4–7,13,20] Second, they were found more frequently in lungs rather than in other organs.[3,13,14] Third, postmortem studies revealed TE in most patients, which presented diverse organisational stages indicating a persistent local hypercoagulable state rather than a single embolic episode.[15,17,26] Lastly, contrary to what occurs in non-COVID-19 patients, thromboprophylaxis may not be as effective in preventing TE in COVID-19.[6,20,27–29]

4.1 | Previous Studies

The association between lung opacification areas and TE in COVID-19 has been explored previously in few studies with heterogeneous results[6,13,20,21] and contradictory data.[30] This relationship is not clear yet, since in all studies, CTPA was mostly performed when TE was clinically suspected, which is challenging because of the overlapping symptomatology of moderate–severe COVID-19 and TE. Therefore, only those patients with a more severe condition and/or with classical TE clinical spectrum may have been included. Furthermore, all these studies were retrospective, most of them with a very small sample size,[13,20,30] and they just analysed the presence of pulmonary involvement in lung segments, but the nearby lung parenchyma around thrombi was not. In the present study, we assessed for the first time the presence of lung opacification 10 mm away or less from each thrombus. We found a significantly higher percentage of TSO than TLI in consecutive hospitalised COVID-19 patients, regardless of clinical suspicion.

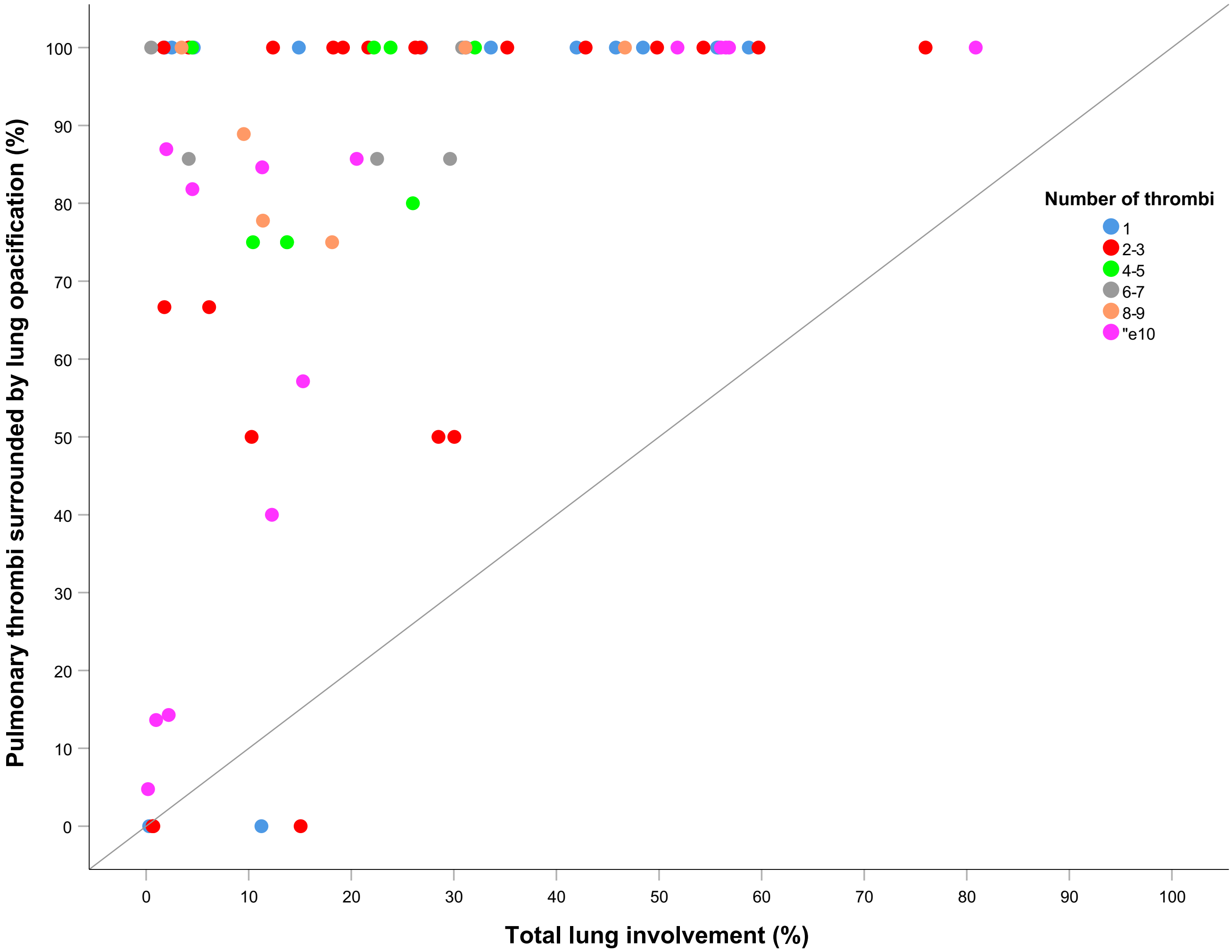

FIGURE 3 Scatter graph analysis of the relationship between the TLI and the PTSO in each patient.

Similarly, other studies showed that thrombi were predominantly located in areas with parenchymal opacification. However, this could be justified by high rates of TLI (circa 50%) in these patients,[6,13,20,21] and an adjustment for the proportion of lung consolidation was not performed. Thus, we found that percentage of TSO was clearly higher than the expected random distribution of thrombi within areas with pulmonary consolidation in relation to the proportion of affected lung in each patient (Figure 3). In addition, a greater predominance of TSO was observed, both when stratifying by TLI (Figure 4) and independently of the NT or whether they were located proximal or distal within the pulmonary arteries. We consider that all these findings highlight the close relationship between thrombosis and lung opacifications in COVID-19.

4.2 | Potential mechanisms

Although our study does not intend to evaluate pathogenic mechanisms, it could be stated that several pathophysiologic changes related to COVID-19 might promote local lung thrombi formation through various pathways. Immunothrombosis is the process by which inflammation activates coagulation leading to microthrombi formation to isolate microorganisms and has been showed to play a key role in the physiopathology of COVID-19.[18] Furthermore, numerous signalling pathways and cytokines have been described to play a role in the mechanisms of hyper-inflammation and endotheliopathy.[31] We compared laboratory findings, inflammatory profile and pulmonary embolism biomarkers according to the percentage of pulmonary thrombi surrounded by lung infiltration to study potential pathophysiological factors in more detail. IL-10 levels, among others, have been found high and predict poor outcomes.[32] We notably found that IL-10 was upregulated in those patients who had all TSO, thus it could represent an alternative novel mechanism promoting in situ PT in COVID-19. In the setting of immunothrombosis, platelets show an increased responsiveness that promote endothelial dysfunction,[33] and increments in the proportion of immature

TABLE 2 Baseline laboratory data according to the percentage of pulmonary thrombi surrounded by lung infiltration.

Percentage of pulmonary thrombi surrounded by lung infiltration	None ($n=5$)	Some thrombi ($n=24$)	All thrombi ($n=41$)	p Value
Blood count, baseline				
Haemoglobin, g/dL	13 (13–14)	14 (13–15)	14 (13–15)	.81
Leucocyte count, $10^3/\mu L$	12 (8–13)	8 (6–12)	8 (6–12)	.77
Neutrophil counts, %	84 (77–87)	82 (73–86)	81 (75–85)	.70
Biochemical profile, baseline				
Glucose, mg/dL	165 (116–190)	140 (113–187)	132 (110–153)	.82
ALT, U/L	36 (27–41)	33 (19–47)	31 (22–48)	.92
Urea, mg/dL	48 (44–53)	38 (26–57)	36 (32–54)	.33
Creatinine, mg/dL	.8 (.7–1.0)	.9 (.8–1.2)	.9 (.8–1.2)	.68
Sodium, mEq/L	139 (137–140)	137 (134–140)	137 (135–139)	.73
Potassium, mEq/L	4.6 (4–4.7)	4.2 (3.8–4.6)	4.1 (3.8–4.4)	.63
Cholesterol, mg/dL	116 (111–128)[a,b]	167 (127–227)	153 (114–178)	**.03**
Triglyceride, mg/dL	135 (120–165)	123 (106–185)	161 (114–218)	.38
Coagulation function, baseline				
PT, %	77 (76–86)	82 (67–87)	79 (65–86)	.90
INR	1.2 (1.1–1.2)	1.1 (1.1–1.3)	1.1 (1.1–1.3)	.99
Fibrinogen, mg/dL	777 (723–856)	792 (616–899)	639 (431–790)	.05
D-dimer, ng/mL	687 (543–1892)	2129 (508–5350)	522 (290–4474)	.28
Arterial blood test, baseline				
PaO_2/FiO_2 ratio	276 (257–276)	295 (246–333)	250 (202–302)	.43
pH	7.50 (7.48–7.52)	7.46 (7.44–7.51)	7.46 (7.45–7.48)	.20
PaO_2, mmHg	58 (56–62)	68 (53–76)	82 (53–80)	.67
$PaCO_2$, mmHg	28 (26–30)	31 (27–34)	33 (30–37)	.10

Note: Values represent median (IQR). Bold indicates statistically significant differences between the three groups.

Abbreviations: ALT, alanine aminotransferase; FiO2, fractional inspired oxygen; PaCO2, partial pressure of arterial blood carbon dioxide; PaO2, partial pressure of arterial blood oxygen; PT, prothrombin time.

[a]Indicates statistically significant differences between patients with no thrombi surrounded by pulmonary opacification vs. patients with some thrombi surrounded by pulmonary opacification.

[b]Indicates statistically significant differences between patients with no thrombi surrounded by pulmonary opacification vs. patients with all thrombi surrounded by pulmonary opacification.

platelets, which can be detected with PDW values, and which were found to be higher in patients with all TSO when compared to patients with only some TSO in the present study. Remarkably, PDW has also been found higher in non-COVID-19 TE,[34] and it has been described both as a severity marker[35] and as a predictive variable of TE in COVID-19.[5] Moreover, other additional factors implicated in pathogenesis of COVID-19, including old age, diabetes mellitus, obesity and hypoxic environment, can further induce platelet activation, leading to increased immunothrombosis.[31]

Previous studies,[6,13,20,21] as well as data from the present study, have shown that most thrombi in COVID-19 were in vessels that were surrounded by opacifications, which may trigger thrombosis by complex, multifactorial, interrelated and still largely unclear mechanisms. However, 24.9% of thrombi were observed in non-consolidation zones, which could represent a different phenotype of PT and the formation of which may be secondary to a combination of a prothrombotic state triggered by systemic inflammatory response and/or classical embolisation from DVT.

4.3 | Clinical implications

A high incidence of TE has been described despite the patients being on prophylactic heparin.[4–6,20] It has been reported that heparin, in addition to its anticoagulant effect, can reduce both inflammation and SARS-CoV-2 infection properties,[36] and it may induce improvements of endothelial function.[37] Recent randomised trials and

TABLE 3 Inflammatory profile and pulmonary embolism biomarkers according to the percentage of pulmonary thrombi surrounded by lung infiltration.

Percentage of pulmonary thrombi surrounded by lung infiltration	None ($n = 5$)	Some thrombi ($n = 24$)	All thrombi ($n = 41$)	p Value
LDH				
Baseline, U/L	301(286–340)	329,5 (280,0-403,5)	411,0 (310,0-508,0)	.10
Peak, U/L	360 (340–413)	408 (311–507)	493 (350–600)	.08
CRP				
Baseline, mg/dL	5.3 (5.0–7.4)	8.9 (4.3–13.4)	15.4 (4.3–22.2)	.07
Peak, mg/dL	6.9 (5.3–7.4)[a,b]	13.4 (8.9–23.4)	18.2 (13.8–26.5)	**.01**
ESR				
Baseline, mm/h	86 (30–92)	65 (52–91)	72 (44–88)	.97
Peak, mm/h	86 (53–92)	76 (62–94)	76 (67–101)	.89
D-dimer				
Baseline, ng/mL	687 (543–1892)	2129 (508–5350)	522 (290–4474)	.28
Peak, ng/mL	1892 (1130–2034)[a,b]	2961 (2394–6077)	4591 (2845–9312)	**.01**
Ferritin				
Baseline, ng/mL	186 (132–800)	494 (241–1103)	696 (439–1046)	.15
Peak, ng/mL	262 (216–1422)	835 (268–1681)	1107 (525–2432)	.18
Platelet count				
Baseline, 10^3/μL	252 (238–320)	254 (179–303)	191 (156–287)	.27
Peak, 10^3/μL	351 (288–481)	475 (376–508)	341 (287–497)	.33
Lymphocyte count				
Baseline, %	10.8 (8.6–15.9)	10.6 (8.1–15.8)	11.6 (7.3–16.5)	.93
Peak*, %	6.5 (5–8.6)	7 (5–11.4)	5.6 (3.7–8)	.53
NLR				
Baseline	7.8 (4.9–10.2)	7.9 (4.6–10.2)	7.1 (4.6–11.5)	.88
Peak	12.8 (10.2–18)	12.4 (7.5–17.9)	15.8 (10.9–24.9)	.54
PLR				
Baseline	380 (304–472)	247 (194–322)	219 (151–366)	.31
Peak	846 (598–1272)[a]	404 (232–540)	434 (315–712)	**.04**
RDW, %				
Baseline	13 (12.7–13.1)[a,b]	12.1 (11.6–12.8)	12.2 (11.9–12.7)	**.04**
Peak	14.1 (13.1–16.2)	12.8 (12–13.7)	13.1 (12.5–13.8)	.14
PDW, %				
Baseline	16.7 (16.1–17.1)	16.5 (16–16.8)	16.7 (16.2–17.2)	.27
Peak	17.5 (17.1–17.8)	17 (16.8–17.9)	17.6 (17.2–18.3)[c]	**.04**
IL-6, pg/mL peak	72.3 (65.1–81.8)	64 (41–123.6)	57.9 (22.5–177)	.72
IL-10, pg/mL peak	3.7 (1.7–26.8)	4.3 (1.3–8.4)	8.1 (4.1–13.7)[c]	**.05**
NT-pro BNP, pg/mL peak	573 (391–797)	248 (127–736)	199 (110–673)	.28
hs Troponin I, ng/L peak	9.8 (4.1–28.3)	8.9 (3.7–25.3)	10.9 (4.2–34.9)	.44
Fibrinogen, mg/dL peak	777 (723–856)	844 (676–1019)	946 (752–1050)	.37

Note: Values represent median (IQR). Baseline, first variable value; Peak, maximum value; Peak*, minimum value. Bold indicates statistically significant differences between the three groups.

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; NT-proBNP, N-terminal pro hormone B-type natriuretic peptide; PDW, platelet distribution width; PLR, platelet lymphocyte ratio; RDW, red blood cells distribution width.

[a]Indicates statistically significant differences between patients with no thrombi surrounded by pulmonary opacification vs. patients with some thrombi surrounded by pulmonary opacification.

[b]Indicates statistically significant differences between patients with no thrombi surrounded by pulmonary opacification vs. patients with all thrombi surrounded by pulmonary opacification.

[c]Indicates statistically significant differences between patients with some thrombi surrounded by pulmonary opacification vs. patients with all thrombi surrounded by pulmonary opacification.

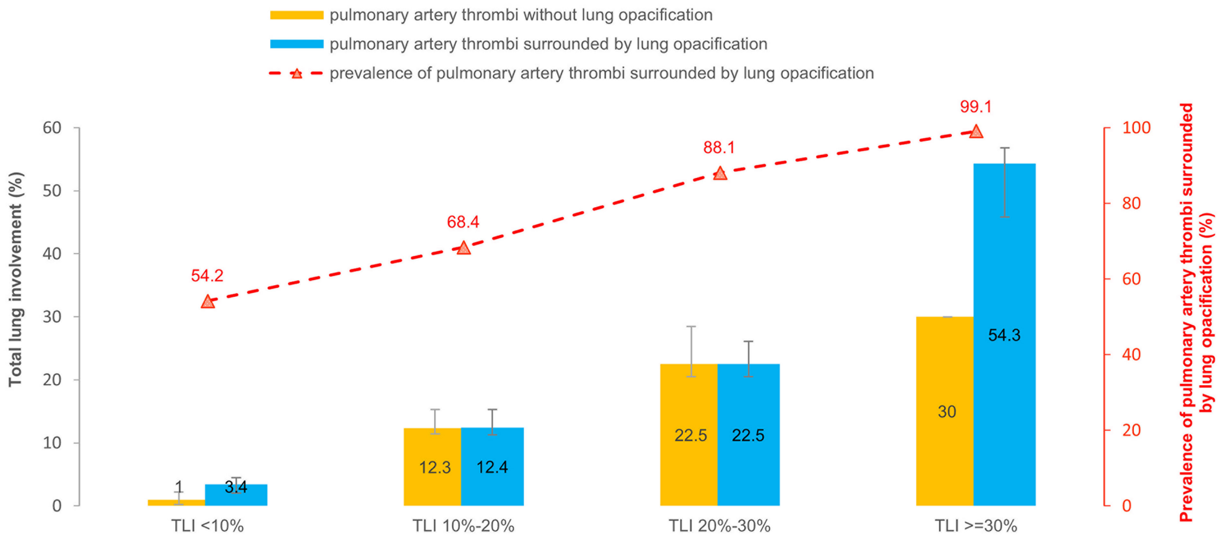

FIGURE 4 Analysis at thrombi level of percentage of artery thrombi surrounded by lung opacification disaggregated by total lung involvement (TLI).

treatment guidelines recommend the use of full anticoagulation with heparin for patients with D-dimer levels above the upper limit of normality who require low-flow oxygen and who do not have an increased risk of bleeding, because of its beneficial effects on TE incidence and on the prognosis of COVID-19.[28,29,38] However, a prophylactic dose of heparin in those patients admitted at ICU is recommended.[38] It is worth mentioning that there was no protocol-specified screening for TE in most of these clinical trials, and their diagnosis was based only on the complicated and confusing clinical suspicion. Moreover, incidental thromboses were less likely to be investigated in patients treated with therapeutic dose of heparin rather than the standard prophylactic anticoagulation dose.

Our patients were selected and studied before these recommendations. All patients except one were on standard or intermediate thromboprophylaxis enoxaparin doses, but only 7 of them required low-flow oxygen during hospitalisation.

From our point of view, the results of our manuscript have some clinical consequences. First, thrombi are quite frequent despite the use of thromboprophylaxis in COVID-19 pneumonia. Second, our results may represent the effects of local immunothrombosis phenomena, and they could be a partial explanation for why standard thromboprophylaxis may not prevent PT. Finally, further studies are needed to clarify the mechanisms between inflammation and PT, as well as to explore new strategies to reduce the thrombotic risk through immunothrombosis control, such as tissue factor expression and inflammasome inhibition. In the meantime, full anticoagulation treatment should be considered according to clinical guidelines and recommendations, by assessing an individualised risk-benefit.[39]

Some strengths of the present study are its prospective enrolment with clear inclusion/exclusion criteria and the intensive characterization from the clinical, laboratory examinations and imaging point of view (including artificial intelligence, as well as the evaluation of the presence of lung opacification surrounding each thrombus by two different radiologists). Furthermore, this is the first prospective study showing a high percentage of TSO in COVID-19 with TE, regardless of clinical suspicion, and that has been adjusted for the lung involvement. Yet, as in any study, there are some potential limitations that deserve some considerations. First, this study lacks a control group with patients who did not have COVID-19. However, TE among patients with non-COVID-19 pneumonia who were admitted to hospital has been reported to be infrequent in a recent retrospective study (72/4304),[40] and a very large study should be performed in such population. Second, this study was carried out before the Omicron variant had become the dominant form and with low vaccination rates, all of which may have had an impact on the thrombi characteristics. Third, D-dimer >1000 ng/mL is a risk factor for TE[5] and death[41] among hospitalised patients, and for that reason, CTPA was only routinely performed to those patients, consequently, the findings among the remaining patients are unknown. Fourth, the sample size was relatively small. Nevertheless, the number of individuals included in the study was sufficiently powered to demonstrate significant differences, and although 71 patients with TE were included, the analysis at thrombi level involved the evaluation of a total of 398 thrombi. In addition, most previous studies evaluating the association between lung opacification areas and PT in COVID-19 included smaller sample size.[13,20,30] Finally, lower limb doppler ultrasound was not performed, so we cannot rule out DVT in our patients.[3,5,15] Some previous studies showed variable rates of DVT, which was increased to 14.7% when ultrasound screening was performed,[42,43] while others concluded that there was a much lower incidence of DVT, reaching a maximum of 2%, despite a high rate of PT (10.1%).[3,44] These contradictory data could be related to the heterogeneity of methodologies, diverse included populations, as well as different proportion of patients on thromboprophylaxis. It must also be considered that most DVT cases were asymptomatic and distally located[43,44] which is known as less likely to be complicated with TE than a proximal DVT. However, even if our patients had DVT, this would not explain why thrombi were located mainly in areas with pulmonary opacifications.

In conclusion, we found that thrombi in COVID-19 pneumonia complicated with TE were located within lung opacities in a higher proportion than total lung involvement, regardless of the percentage of pulmonary infiltrates and clot location. All of this supports the hypothesis that thrombi were mostly located in the lung zones with opacification, suggesting that COVID-19 could generate local pro-thrombotic phenomena secondary to pulmonary infection-inflammation, leading to in situ PT formation rather than "classical TE". Further studies are needed to better clarify and characterise PT in COVID-19 to elucidate the mechanisms and the complex interrelationships between inflammation and PT, which could be relevant to decrease uncertainties about the best thrombo-prophylaxis and PT treatment scheme in COVID-19.

AUTHOR CONTRIBUTIONS

Author contributions: CS, AAF, ESLL, NT and JS conceptualized, administered and supervised the study. AAF designed the study protocol with the help of NTP, and AM. AM and AAF analysed the data. Funding acquisition was the responsibility of AAF and BN. NC, LP, LR, JM, DM, AI and JBR contributed to patient recruitment and data collection. All authors were involved in further drafts of the manuscript and revised it critically for content. All authors approved the final version of the manuscript. The corresponding author attests that all listed authors meet authorship criteria.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest in our publication.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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