

## RESEARCH ARTICLE

# Vascular injury in macroscopically normal skin of patients with severe COVID-19 infection: clinical-pathologic correlations

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**Objectives:** Taking into account that the documentation of the histopathological features in severe disease caused by SARS-CoV-2 has been scarce due to the avoidance of performing autopsies, the aim of the study was to detect the microscopic changes associated with severe COVID-19 infection in normal-appearing skin, without prominent dermatologic signs of a generalized microvascular thrombotic disorder, in accordance with the clinical evolution of disease. **Methods:** In this morphological and immunohistochemical study we included cutaneous biopsy samples from 12 symptomatic patients with severe and critical type SARS-CoV-2 infection (with the admission date between February and June 2020), treated in the Intensive Therapy Unit Care of Emergency County Hospital Targu-Mures, Romania. **Results:** The average age of our patients was  $65.18 \pm 14.21$  years (range 41 to 83), and 66.67% of the patients were male. The histological and immunohistochemical assessment of cutaneous biopsies: in 4 cases the histological examination revealed small fibrin thrombi in deep-seated venules and small veins of subcuticular adipose tissue, and also 4 cutaneous biopsies showed occlusive vascular thrombosis in association with massive perivascular inflammatory infiltrate destroying and compromising the integrity of the vessel wall. The immunohistochemical examination of the composition of perivascular inflammatory infiltrate showed a predominance of CD3 positive lymphocytes, admixed with CD68 positive Mo/MF, some of them activated with FXIII expression. In the perivascular infiltrate, the presence of granulocytes and B lymphocytes was not characteristic. **Conclusion:** According to our observations, in severe COVID-19, the cutaneous tissue is involved even in the absence of clinically obvious changes. Due to the relatively easy accessibility of skin samples, these could be applied to determine the severity of the patient's clinical status, and to predict the necessity for anti-complement or anticoagulant treatments in the early stages of a severe SARS-CoV-2 infection.

**Keywords:** COVID-19, skin histopathology, vascular injury, thrombosis, endothelial cell injury, perivascular inflammatory infiltrate

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## Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of Coronavirus disease 2019 (COVID-19) has been identified as the causal agent of severe pneumonia in Wuhan City, Hubei Province, China. Since December 2019, the virus has spread worldwide, causing thus far more than 16,540,137 diseases and more than 655,300 deaths worldwide [1]. The pathogenesis of this disease is only partially known and at this moment there is no specific treatment for eradication of the virus in those infected. The virus can enter the human body through its angiotensin 2 convertase receptor (ACE-2), which is characteristic for various organs thus facilitating viral entry into targeted cells. The attachment of the enveloped, non-segmented, positive sense RNA virus to the ACE-2 receptor is mediated by the so-called spike or S transmembrane glycoprotein. Due to the fact that ACE-2 receptors are found in the lower respiratory tract epithelium, especially in type II alveolar cells (AT2), the predominant target organs are the lungs. They are also present in other cells, such as absorptive enterocytes and cholangiocytes, myocardial cells, proximal tubular epithelial cells, urothelial cells [2], the basal layer of the epidermis, endothelial cells of dermal blood vessels and

eccrine adnexal tissue [3]. In this context, a multi-organ disease may develop in infected patients, due to the rapid spread of the infection that can affect the kidneys, nervous and cardio-vascular systems, clotting pathways, skin and the immune system in some patients. According to statistics, not all contacts get sick (many remain asymptomatic) and not all patients develop respiratory failure [4]. From an epidemiological point of view, it is important to emphasize that the asymptomatic infections have the same infectivity as symptomatic infections [5]. How the body responds to the infection depends on several factors, of which age and body condition may play an important role in the severity of COVID-19. This is related to different immune responses and other potential pathogenesis. The host response to SARS-CoV-2 is closely similar to severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), being mediated by cytokines [6]. The immune response induced by SARS-CoV-2 infection seems to be biphasic: during the incubation and non-severe stages, a specific adaptive immune response is required to eliminate the virus and to preclude disease progression to severe stages. In case of an inadequate immune response, the virus propagates and causes a massive destruction of affected tissues, particularly those with high ACE-2 expression. At this stage, an over-activated immune system via excessive inflammation leads to high levels of

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cytokines (“cytokine storm”) [7,8]. Cytokines are at the core of the pathophysiology of COVID-19; while some of them are beneficial (type-I interferon, interleukin-7), others appear detrimental (interleukin-1 $\beta$ , -6, and TNF- $\alpha$ ) particularly in the context of a strong pro-inflammatory cytokine storm [9].

Recent studies have demonstrated an extensive tendency for coagulopathy, thrombosis, micro-thrombosis, and disseminated intravascular coagulation due to severe COVID-19, possibly representing a peculiar pathogenic manifestation of viral sepsis [10,11].

In addition to fever, respiratory symptoms, cardio-vascular and gastrointestinal symptoms and anosmia, several studies reported skin manifestations of COVID-19 [12], Kaya and al. summarized the skin lesions described in 57 recent articles thus far, that affect between 1.8% and 20.4% of COVID-19 patients and concluded that skin lesions are highly varied (maculopapular eruptions, acral areas of erythema with vesicles or pustules (pseudo chilblain), urticarial lesions, other vesicular eruptions and livedo or necrosis). They may not be related to the severity of the condition and resolve spontaneously in a few days. Corresponding of clinical manifestations, the histopathological features of these lesions also vary, from epidermal and dermal vascular lesions up to microvascular injury and thrombosis (in severe cases) [13]. Focusing on specific skin changes, Galván et al. classified cutaneous features into 5 categories based on their morphology: (1) pseudo-chilblain, (2) vesiculobullous, (3) urticarial lesions, (4) maculopapular eruptions and (5) livedo or necrosis, the latter being suggestive for vaso-occlusive disease and could warrant a rapidly progressive/life-threatening disease [14]. A single article reported histological aspects of purpuric skin involvement in 3 severely ill COVID-19 patients, (skin biopsies from gluteal region, palms and soles, chest and limbs) parallel with histological evaluation of normal-appearing deltoid skin and has illustrated that both the affected and the apparently normal skin, revealed thrombogenic vasculopathy and deposits of C5b-9 and C4d complement and that critically ill patients can suffer thrombotic microvascular injuries involving the lungs, skin and possibly other organs [15].

Taking into account that the documentation of the histopathological features in severe disease caused by SARS-CoV-2 has been scarce due to the avoidance of performing autopsies, the aim of the study was to detect the microscopic changes associated with severe COVID-19 infection in normal-appearing skin, without prominent dermatologic signs of a generalized microvascular thrombotic disorder (DIC), in accordance with the clinical evolution of disease.

## Material and methods

### Study design and patients

In this morphological and immunohistochemical study we included cutaneous biopsy samples from 12 symptomatic patients with severe and critical type SARS-CoV-2 infection (with the admission date between February and June

2020), all confirmed by positive tests for SARS-CoV-2 in a reverse transcriptase polymerase chain reaction assay and treated in the Intensive Therapy Unit Care of Emergency County Hospital Targu-Mures, Romania, for acute respiratory distress syndrome. All of the patients had severe or critical type disease, based on clinical characteristics [16].

Biopsies were performed immediately after admission by surgeons specialized in vascular surgery, performed under local anesthesia (1% lidocaine) and the conditions established by the hospital protocols and regulations, from the lower third of the lateral face of the calf. An elliptical incision of approximately 1 cm centered on a superficial venous branch was performed. The study was conducted with the permission of the Biomedical Research Ethics Committee of Emergency County Hospital Targu-Mures Romania (approval no.Ad.11420), and the declaration of Helsinki. Prior to surgery, each patient has signed an informed consent in relations with the surgical intervention, enrollment in the study and publication of scientific data.

### Microscopic examination and immunohistochemistry

For the assessment of morphological changes, tissue samples were processed according to standard histological procedures. Serial sections of 4-5  $\mu$ m were performed; one section was stained with hematoxylin and eosin (H&E), and adjacent sections were selected for immunohistochemistry. The morphological modifications were established on the H&E stained sections. We focused on the changes involving the epidermal/dermal interface, dermal and hypodermal structures including also small veins situated in the subcuticular adipose tissue. We separately evaluated the non-vascular changes from the vascular injuries (perivascular inflammatory infiltrate/thrombosis/endothelial cell injury) at the level of the examined structures. All sections that showed lymphocytic infiltrate associated or not with vascular injury were immunostained to establish the immunological profile of inflammatory cells (neutrophil granulocytes, macrophages and T-cells). The following leukocyte markers were used, in compliance with the protocol proposed by the manufacturer for each antibody: anti-CD15 clone Carb-3 (Dako), anti-CD68 clone ab955 KP1 (Abcam), anti-CD3 SP7 ab 21,703 (Abcam) and anti-Factor XIIIa clone E980.1 (Novocastra Laboratories). Endothelial cell injury with vascular thrombosis has been confirmed using anti-Factor VIII (von Willebrand). Endothelial discontinuity was considered a sign of endothelial cell injury. As for the secondary antibody, we used EnVision™ FLEX/HRP (Dako) in combination with a 3,3'-diaminobenzidine (DAB) solution for the chromogenic identification of the above-mentioned antigens. As negative control, the primary antibodies were substituted with normal serum to avoid nonspecific staining. Tissue examination was followed by a detailed evaluation of the morphological changes in all three layers of the skin focusing on the microvascular injury. Clinical and laboratory data were reviewed and the correlation with the histology data has been made.

**Statistical analysis**

We performed statistical analysis with Graphpad Prism 7. Patients with divergent histopathological signs were compared by the Chi-square test and Fisher’s exact test to assess the between-group statistical differences. A p-value < 0.05 was considered significant.

**Results**

**Baseline characteristics**

The average age of our patients was 65.18 ± 14.21 years (range 41 to 83), and 66.67% of the patients were male. The clinical data of these patients (including demographic, baseline characteristics, symptomatic and radiological characteristics, laboratory data on admission) and the evolution

(deaths, recoveries) are reported in Table I and Table II. The average duration of hospitalization was 11 days (ranging between 3 and 23). Initially, at the time of admission, the clinical symptoms were dominated by acute respiratory failure (respiratory distress, cough, fever) and severe hypoxemia (SpO<sub>2</sub> below 80%), associated with tachycardia and fever (two of them with altered mental status). Acute unilateral or bilateral exudative lesions were identified on radiographic images (ground glass opacity, nodular opacifications and pleural effusion). Co-existing respiratory pathogens were isolated in two cases: from patient 1 *Acinetobacter baumannii* and *Rothia mucilaginosa* and *Enterobacter* for patient 7. Other non-respiratory pathogens were identified: patient 1 had urine culture positive for *Enterococcus faecium* and for

**Table I. Laboratory data of 1-6 patients with severe COVID-19 infection**

Patient characteristics	Case series					
	1	2	3	4	5	6
Age	60	69	65	74	42	83
Sex	M	M	F	M	M	M
Onset symptoms	Cough, Fever, Chills	Fever, Acute respiratory distress, Tachycardia	Fatigue, Acute respiratory distress, Tachycardia	Acute surgical abdomen: volvulus, Acute respiratory distress	Altered mental status, Left hemiplegia, Acute respiratory distress	Altered mental status, Motor deficit in the right limbs, Acute respiratory distress
Low SpO <sub>2</sub>	Yes	Yes	Yes	Yes	Yes	Yes
Diagnosis at admission	Acute respiratory distress	Acute respiratory failure	Acute respiratory distress	Volvulus, Acute respiratory distress	Patient under treatment for COVID -19 infection suffers an cerebral vascular accident	Cerebral vascular accident, Suspected COVID-19 pneumonia, Mixed aphasia
Comorbidities	Alcoholic liver cirrhosis, Heart failure: cardiomegaly, COPD	Pulmonary, Fibrosis	Post-stroke lacunar status, Hypertension, Atrial fibrillation	Hypertension, Ischemic heart disease, Atrial fibrillation	Hypertension, Ischemic heart disease, Obesity	Hypertension, Ischemic heart disease, Atrial fibrillation
Radiographic findings at admission	Multifocal bilateral basal opacity with small pleural and pericardial effusions	Bilateral postero-basal interstitial opacities	Airspace opacity in a central peribronchovascular distribution (APE), Bilateral patchy opacities	Multifocal bilateral opacities, Abdominal: characteristic signs of sigmoid volvulus	Bilateral Interstitial opacities, Cranial CT: large, ill-defined hypo-attenuating area in the right cerebral hemisphere	Three nodular opacification in right pulmonary Cranial CT: large, ill-defined hypo-attenuating area in the left cerebral hemisphere
Radiographic findings in the evolution of the disease	Widespread bilateral opacities	Ameliorated	Ameliorated	Abdominal: ameliorated; Chest: widespread bilateral opacities	Persistent bilateral Interstitial opacities	Chest: Ameliorated, Extension of brain damage
Anemia (Hb<13 to 14 g/dL)	Yes	No	No	Yes	No	Yes
High WBC (> 10 x 10 <sup>3</sup> /μL)	Yes	Yes	Yes	Yes	Yes	Yes
Lymphopenia (< 1.2 x 10 <sup>3</sup> /μL)	Yes	Yes	No	Yes	Yes	No
Thrombocytopenia (< 150 x 10 <sup>3</sup> /μL)	Yes	Yes	No	No	No	Yes
Elevated creatinine (>1.25 mg/dL)	Yes	Yes	No	No	No	Yes
Elevated CRP (>5 mg/L)	Yes	Yes	Yes	Yes	Yes	Yes
Elevated PCT (> 0.5 ng/ml)	Yes	No	No	No	No	No
Elevated INR (> 1.14)	Yes	Yes	No	Yes	Yes	Yes
Mechanical ventilation/intubation#	Yes	No	No	Yes	Yes	Yes
No. days of hospitalization	23	10	10	7	3	21
Coexistent respiratory pathogens	<i>Acinetobacter baumannii</i>	No	No	No	No	No
Other pathogens	<i>Enterococcus faecium</i> (urine)	No	No	No	No	No
Discharge diagnosis*/ Cause of death**	**COVID-19 pneumonia, <i>Acinetobacter pneumonia</i> , Sepsis	*COVID-19 pneumonia	*HT induced APE, COVID-19 pneumonia	**COVID-19 pneumonia, surgical post-intervention condition for volvulus	**Cerebral vascular accident, COVID -19 pneumonia	**Cerebral vascular accident, COVID -19 pneumonia

Low SpO<sub>2</sub>: oxygen saturation below 95 %; Hb: haemoglobin; WBC: white blood cell count; CRP:c-reactive protein; PCT: procalcitonin; COPD: chronic obstructive pulmonary disease; CK-MB: creatine kinase isoenzyme MB; # during evolution

patient 10 Clostridium difficile toxin was identified in the fecal material. The more distinctive cases, 3 out of 12, were admitted for cerebral vascular accident (two patients), respectively, one case of acute myocardial infarction.

### Co-morbidities

All of the patients previously mentioned had more than two comorbidities, many of them in various associations: ischemic heart disease (5 cases) associated or not with atrial fibrillation, hypertension (7 cases, 2 of them complicated with post-stroke lacunar status), chronic obstructive pulmonary disease -pulmonary fibrosis (3 cases), metabolic disease (obesity/diabetes) 3 cases, and 2 patients presented

chronic kidney disease. All patients had altered laboratory parameters on admission. A significant number of patients had high WBC (91.67%), elevated levels of C-reactive protein (83.34 %), elevated creatinine (58.34 %) and International Normalized Ratio (INR) (83.34 %), but higher levels of procalcitonin were uncommon (25 %). Also, most patients had anemia (50 %), lymphopenia (58.34 %) and thrombocytopenia (41.67%).

Concerning the clinical evolution, 9 patients had unfavorable clinical evolution (75%). Compared with the three patients who had a favorable evolution, the deceased patients (nine) presented more than three significant comorbidities.

Table II. Title

Patient characteristics	Case series					
	7	8	9	10	11	12
Age	75	57	73	81	41	66
Sex	M	M	M	F	F	F
Onset symptoms	Cough, Fever, Acute respiratory distress	Fatigue, Dyspnoea, Polyuria, Polydipsia	Fatigue, Dyspnoea, Acute respiratory distress	Cough, Fever, Fatigue, Dyspnoea, Shortness of breath	Dyspnoea, Shortness of breath, thoracic constriction, precordial pain	Fatigue, Dyspnoea, Cough, Acute respiratory distress
Low SpO <sub>2</sub>	Yes	No	Yes	Yes	No	Yes
Diagnosis at admission	Acute respiratory distress	Acute respiratory distress, Diabetes	Acute respiratory distress	Acute respiratory distress	Suspected COVID-19 pneumonia, Acute subendocardial myocardial infarction (Elevated CK-MB, EKG)	COVID-19 pneumonia
Comorbidities	Hypertension, Atrial fibrillation, Prostate carcinoma, Hepatitis C infection	Diabetes	Hypertension, Peripheral artery disease, endarterectomy of femoral artery of left lower limb, COPD	Hypertension, Ischemic heart disease, Atrial Fibrillation, NYHA II, Post-stroke lacunar status, Chronic kidney disease	End-stage renal disease under dialysis	Hypertension, Ischemic heart disease, Obesity, Medical pass History: COVID-19, pneumonia
Radiographic findings at admission	Multifocal right basal opacity with medium pleural effusions	Bilateral patchy opacities of inferior lobes, Left apical calcified nodule	Bilateral nodular opacifications with massive pleural effusions, Low lung volume, Pulmonary fibrosis	Widespread bilateral opacities	Right pleural effusion, bibasilar patchy condensations	Widespread bilateral opacities
Radiographic findings in the evolution of the disease	Widespread bilateral opacities	Ameliorated	Widespread bilateral opacities associated with pleural effusion	Complete bilateral opacification, Pleural effusion	Ameliorated	Ameliorated
Anemia (Hb<13 to 14 g/dL)	Yes	No	No	Yes	Yes	No
High WBC (> 10 x 10 <sup>3</sup> /μL)	Yes	Yes	Yes	Yes	Yes	No
Lymphopenia (Lymphocytes < 1.2 x 10 <sup>3</sup> /μL)	Yes	No	Yes	Yes	No	No
Thrombocytopenia (Thrombocytes < 150 x 10 <sup>3</sup> /μL)	Yes	No	No	Yes	No	No
Elevated creatinine (>1.25 mg/dL)	Yes	Yes	No	Yes	Yes	No
Elevated CRP (>5 mg/L)	Yes	No	Yes	Yes	No	Yes
Elevated PCT (> 0.5 ng/ml)	Yes	No	No	Yes	No	No
Elevated INR (>1.14)	Yes	No	Yes	Yes	Yes	No
Ventil	Yes	No	Yes	No	No	No
Number of days of hospitalization	7	9	16	20	14	7
Coexistent respiratory pathogens	Rothia Mucilaginoso Enterobacter	No	No	No	No	No
Other pathogens	No	No	No	Clostridium difficile	No	No
Discharge diagnosis* or Cause of death**	**COVID -19 pneumonia, sepsis	**COVID-19 pneumonia, Diabetes	**COVID -19 pneumonia, surgical post- intervention condition for endarterecto-my	**COVID -19 pneumonia, Cerebral vascular accident, Sepsis	* Acute subendocardial myocardial infarction, COVID -19 infection	*COVID -19 pneumonia

Low SpO<sub>2</sub>: oxygen saturation below 95 %; Hb: haemoglobin; WBC: white blood cell count; CRP:c-reactive protein; PCT: procalcitonin; COPD: chronic obstructive pulmonary disease; CK-MB: creatine kinase isoensyme MB

**The histological and immunohistochemical assessment of cutaneous biopsies**

On the H&E stained sections, 2 of the skin biopsy samples showed no epidermis/dermis/hypodermis and vascular alteration (patients 3 and 10). In 2 skin samples (patients 2 and 8) we found mild modifications represented by isolated foci with interface dermatitis, moderate perivascular lymphocytic infiltrate, involving the vessels in the superficial and the deep dermis (Figure 1A). In 4 cases (patients 1, 4, 7 and 11) the histological examination revealed small fibrin thrombi in deep-seated venules and small veins of subcuticular adipose tissue (Figure 1B), and also 4 cutaneous biopsies (patients 5, 6, 9 and 12) showed occlusive vascular thrombosis (Figure 1C-D) in association with massive perivascular inflammatory infiltrate destroying and compromising the integrity of the vessel wall. (Figure 1E), all of them with endothelial cell injury (endothelial cell swelling and cell detachment) (vWF/ DAB ) (Figure 1F ). The immunohistochemical examination of the composition of perivascular inflammatory infiltrate showed a predominance of CD3 positive lymphocytes (Figure 1G), admixed with CD68 positive Mo/MF (Figure 1H), some of them activated with FXIII expression (Figure 1I). In the perivascular infiltrate, the presence of granulocytes and B lymphocytes was not characteristic.

Regarding the correlation of histological, clinical and laboratory data we found that all patients with lymphopenia (7 cases with mean age 65.57 years) were diagnosed with severe bilateral pneumonia and had an unfavorable clinical evolution (six of them died). In addition, in their skin biopsies samples, fibrin and occlusive thrombi were constantly present (excepted case 2). Statistical analysis showed that the presence of vascular lesions (perivascular infiltrate, microthrombosis, occlusive thrombi and en-

dothelial injury) did not affect survival, the latter is also unrelated to thrombocytopenia.

**Discussion**

Clinical experience over the past six months has confirmed that morbidity and mortality of COVID-19 are predominantly linked to elderly age, to male gender and cumulative comorbidities (as hypertension, heart failure, chronic kidney disease under dialysis, diabetes, obesity, cancer, chronic obstructive pulmonary disease) leading to multisystem organ failure, followed by intensive care unit admission and need for mechanical ventilation or tracheal intubation. [17]. The mechanisms responsible for the high morbidity and mortality in these patients are not sufficiently elucidated, and confusion is generated due to the absence of histopathological studies performed on an adequately high number of patients as a consequence of the avoidance of performing autopsies.

In spite of the fact that the pathophysiology is relatively well mapped, at the moment there is no specific treatment for virus eradication. To date, therapeutic management of patients with COVID-19 is only limited to symptomatic or palliative treatment depending on the severity of the disease [18].

The majority of severe COVID-19 cases are frequently associated with micro- and macro-thrombosis, in both, the microcirculation and venous/arterial circulations, particularly in patients who require admission to intensive care unit, dominated by respiratory complications (pneumonia/adult respiratory distress syndrome). However, the spectrum of thrombotic events is wide, including in situ pulmonary thrombosis, deep-vein thrombosis with associated pulmonary embolism, as well as arterial thrombotic events (stroke, myocardial infarction, limb artery throm-

**Table 3. Microscopic and immunohistochemical analysis of 12 skin biopsies**

Cases	Skin biopsies			
	Epidermis/dermis/hypodermis	Vascular injury		
		Perivascular inflammatory infiltrate	Thrombosis	Endothelial cell injury
1	Superficial vascular ectasia	Massive: CD3 positive lymphocytes and CD68 Mo/MF (superficial dermis)	Dermal -hypodermal interface: venules with small fibrin thrombi	Yes
2	No alterations	Moderate: predominantly CD3 positive lymphocytes (superficial dermis)	Absent	No
3	No alterations	absent	Absent	No
4	Interphase dermatitis	Massive: CD3 positive lymphocytes , rare CD68 Mo/MF (dermis and hypodermis)	Small fibrin thrombi in venous lumen	No
5	extravasated red blood cells in the hypodermis	Absente	Occlusive thrombi in the deeper reticular dermis, fibrin thrombi in subcutis	Yes
6	No alterations	Massive: CD3 positive lymphocytes, CD68 and FXIII positive Mo/MF, CD15 (dermis and hypodermis)	Occlusive thrombi in the deeper reticular dermis	Yes
7	No alterations	Massive: CD3 positive lymphocytes, CD68 and FXIII positive Mo/MF and rare CD15 positive granulocytes (dermis and hypodermis)	Small fibrin thrombi in venous lumen	No
8	vascular ectasia and extravasated red blood cells in the hypodermis	Moderate: CD3 positive lymphocytes, FXIII positive Mo/MF and rare CD15 positive granulocytes (dermis and hypodermis)	Absent	No
9	No alterations	Massive, predominantly FXIII and CD68 positive Mo/MF (in the deeper reticular dermis)	Occlusive thrombus	Yes
10	No alterations	Absente	Absent	No
11	Superficial vascular ectasia	Massive, CD3 positive lymphocytes, (dermis and hypodermis)	Fibrin thrombi in venous lumen	Yes
12	No alterations	Massive, CD3 positive lymphocytes and CD68 Mo/MF (dermis and hypodermis)	Occlusive thrombi	Yes

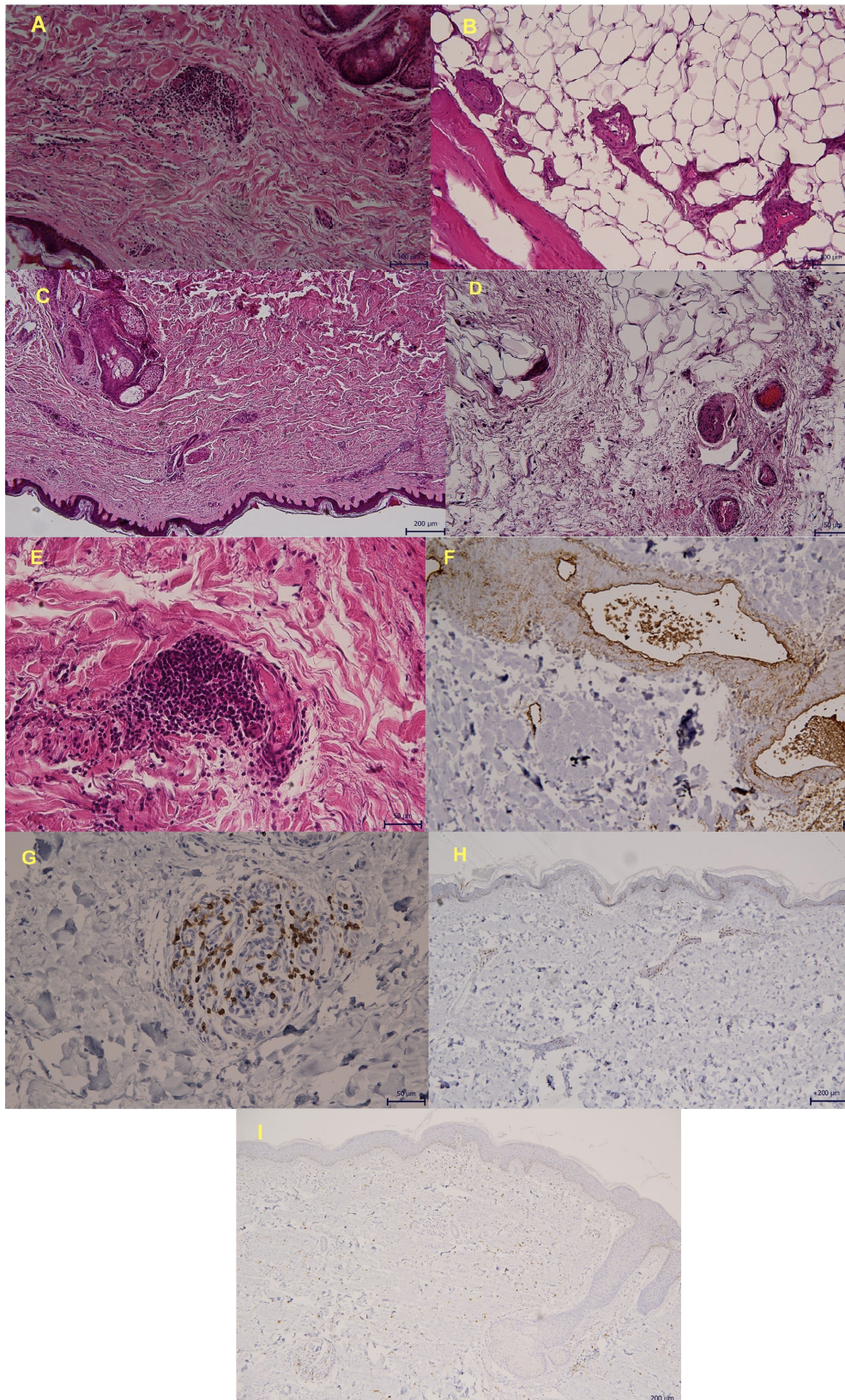


Fig. 1. (A-I): Histopathological and immunohistochemical characterization of skin vascular injury associated with severe COVID-19 disease. (A) Cutaneous biopsy with mild microscopic modifications predominated by moderate perivascular lymphocytic infiltrate involving the vessels within deeper dermis (Hematoxylin and eosin, 200 x). (B) Small fibrin thrombi in deep-seated venules and small veins of subcuticular adipose tissue were observed in third of cases (Hematoxylin and eosin, 200 x). (C-D) Prominent occlusive thrombus in the deeper dermis near the sebaceous gland (Hematoxylin and eosin, 100 x) and in hypodermis (Hematoxylin and eosin, 400 x). (E) A vascular occlusion associated with a massive perivascular inflammatory infiltrate destroying and compromising the vessel wall integrity (Hematoxylin and eosin, 400 x). (F) Vascular ectasia and blood extravasation in the hypodermis due to endothelial injury with endothelial cell swelling and cell detachment (vWF/ 3,3'-Diaminobenzidine, 200 x). (G) Immunohistochemical analysis of perivascular cellular infiltrate in severe COVID-19 disease shows positive CD3 staining around the small dermal blood vessels highlighting the predominance of T lymphocytes (CD3/3,3'-Diaminobenzidine, 400 x). (H) CD68-positive monocytes/macrophages mix between lymphocytes (CD3/3,3'-Diaminobenzidine, 100 x). (I) Some of monocytes express also FXIII (FXIII/3,3'-Diaminobenzidine, 100 x).

bosis) [19,20] The vascular endothelium is considered to be one of the targets for SARS-CoV-2 through the presence of ACE-2 receptors in both arterial and venous endothelial cells (ECs) in all human tissue, including skin. In severe infection, the high levels of pro-inflammatory cytokines (mostly released by macrophages, activated T helper lymphocytes but also by endothelial cells) lead to capillary leak. During infection, endothelial dysfunction and small fibrin thrombi formation is secondary, as a result of complement activation and ECs attack [21]. Activated ECs release von Willebrand factor (vWF) and factor VIII, which contribute to clot formation [22]. In our findings, a morphological aspect in the blood vessels of the skin, corresponding to this activation cascade was the extravasation of red blood cells as a result of capillary leakage, endothelial inflammation with the accumulation of mononuclear cells (T lymphocytes, MO/MF, rare neutrophils). We also observed endothelial cell swelling and cell detachment, along with small fibrin thrombi or occlusive thrombi in the lumen of the arteries and veins, which were well drawn through the von Willebrand Factor labeling. Histological evidence of endothelial dysfunction during COVID-19 infection was described in several post-mortem studies in different organs, similar to skin [23-26]. Some of the authors found viral inclusion structures in ECs, as well as endothelial inflammation with evidence of direct viral infection of the ECs [19,22]. In this context, the endothelial dysfunction can be a therapeutic target in severe COVID-19 infection by blocking the entry of the virus into the ECs, immunomodulatory therapies such as anti-pro-inflammatory cytokines strategies and complement and coagulation cascade blockade [27].

COVID-19 also shows features of pancellular activation. As noted, SARS-CoV-2 particles invade the endothelial cells, producing endotheliitis and perivascular inflammatory response. As vasculitis mimics, we observed an inflammatory response in skin-biopsies histology. The vascular compromise in the majority of cases was accompanied by a pauci- or massive-inflammatory response in the form of a coat-sleeve-like mononuclear infiltrate around the blood vessels with T-lymphocytes predominance, among which MO/MF and neutrophils were admixed.

Several studies have demonstrated the close connection between thrombosis and inflammation, mutually supporting each other in creating a vicious circle, which results a hypercoaguable state [28]. The platelet activation response, with or without associated activation of hemostasis, is followed by a mild thrombocytopenia, often observed in patients with severe COVID-19 [29]. One question that remains constant in most studies showing the clinical course of patients with COVID-19 is that thrombocytopenia may be an predictive marker of microvascular thrombosis or not. It has been previously shown that approximately 20% of patients have mild thrombocytopenia ( $100-150 \times 10^9 / L$ ), our study documented this sign in 41.67% of the cases.

Numerous studies confirmed that the subjects who died of COVID-19 had significantly lower lymphocyte level compared to the survivors [30], especially those who suffered the cytokine storm syndrome. Lymphopenia is characteristic in elderly patients (more than 60 years old), and in those who need therapy in the intensive care unit [31]. During a normal immune response, natural killer (NK) cells and cytotoxic T cells (CTLs) have the ability to eliminate infected cells, whereas T helper lymphocytes adjust the entire adaptive immune response. In SARS-CoV-2 infection, an increasing number of activated circulating T helper lymphocytes and cytotoxic T lymphocytes were detected in the first week, and the high levels of pro-inflammatory cytokines released by activated macrophages, monocytes, neutrophils and other activated cells induce granulopoiesis, and reduce lymphopoiesis in the bone marrow, as a possible causes of lymphopenia [32]. In our study, we found lymphopenia in 75% of patients, associated with *high WBC*, elevated levels of C-reactive protein *and INR*, all of them having severe bilateral pneumonia and multiple organ failure (requiring mechanical ventilation/intubation during evolution), and characteristically unfavorable clinical evolution (non-survivors). Our findings are consistent with data from literature, since advanced age, numbers of affected pulmonary lobes, high leucocyte along with low lymphocyte count, and elevated CRP levels on admission were substantial risk factors associated with death in severe COVID-19 [33-35].

Our report is limited by the number of cases. We acknowledge that the clinical, laboratory and histological results do not characterize the clinical evolution, but it is a cross-section of a narrow spectrum at the moment of admission. However, the presence of small fibrin- and occlusive thrombi along with perivascular inflammation, and endothelial damage in macroscopically healthy skin of those SarS-CoV-2 infected was not reported previously. It is important to stress out that cutaneous vascular lesions with thrombi associated with perivascular inflammatory infiltrate and endothelial cell injury develop only in a subset of severely ill patients with COVID-19. It is likely, therefore, that the tissue response is different in individuals with COVID-19 who are asymptomatic or have only mild symptoms.

## Conclusion

According to our observations, in severe COVID-19, the cutaneous tissue is involved even in the absence of clinically obvious changes. A skin biopsy that shows vascular injury with thrombosis can be interpreted as the activation of the coagulation system associated with COVID-19, and a predictor factor of a rapidly progressive/life-threatening evolution. Due to the relatively easy accessibility of skin samples, these could be applied to determine the severity of the patient's clinical status, and to predict the necessity for anti-complement or anticoagulant treatments in the early stages of a severe SARS-CoV-2 infection.

## Conflicts of interest

The authors declare no competing interests.

## Author contributions

M.V.A: Conceived of the presented idea, developed the theory, coordinated the medical team, supervised the project. C.M.C: Performed the biopsies necessary for the elaboration of this study, contributed to the writing of the study. H.E: designed the study, performed histopathological and immunohistochemical analysis of skin biopsies, contributed to data analysis, performed figure design and provided a critical review of the manuscript and performed last edited form of it. All authors contributed to data interpretation and editing the manuscript.

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35. Low SpO<sub>2</sub>: oxygen saturation below 95 %; Hb: haemoglobin; WBC: white blood cell count; CRP: c-reactive protein; PCT: procalcitonin; COPD: chronic obstructive pulmonary disease; CK-MB: creatine kinase isoenzyme MB; # during evolution