Lungs' Microscopic Patterns of Vessels in Patients Deceased with or by SARS-CoV-2 Infection

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Abstract

Background: Even If Specific mechanisms are not completely understood, several studies highlighted Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) ability to alter vascular homeostasis. In the literature, multiple reports of microscopic pulmonary findings of vascular structures in patients deceased by or with SARS-Cov-2 infection are available. Nevertheless, the scientific literature lacks a systematic analysis of these findings. **Methods**: the authors realized a systematic review of the literature in order to identify common microscopic patterns representative of pulmonary vascular damage: useful data for pathologists in clinical and forensic settings. The research yielded 23 articles (79 total cases). Quali/quantitative analysis was carried out. **Conclusion**: the review allowed to identify vascular thrombosis (especially in lesser caliber vessels) as common microscopic pattern. The recurrence of this pattern was confirmed by scientific literature data which demonstrate SARS-CoV-2 ability to interfere with coagulation cascade. Other meaningful microscopic findings were also discussed, even if their low frequency in study population did not allow to define them as common.

Keywords: *COVID19; endothelium; forensics; lung parenchyma; pathology;SARS-CoV-2; vessels*

Introduction

Even if specific mechanisms are not completelyunderstood, several studies highlightedSevere Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) ability to alter vascular homeostasis[1-4]. Pulmonary vascular damage seems to have a fundamental rolein the aforementioned phenomenon, because vascular structures (especiallythe endothelium) are considered as the anatomical substrate in which coagulative, immune, and inflammatory balances interact[2,5]. Thus, according to the literature,the same vascular damage caused by the virus can simultaneously determine coagulation's over-activation, improper cytokine release (cytokine storm), and immune system alterations. Several authors stated that the latter events constitute foundation for the progression of Coronavirus Disease 2019(COVID19)[2,4].

In the literature, multiple reports of microscopic pulmonary findings of vascular structures in patients deceased by or with SARS-Cov-2 infection are available[1,3,4]. Nevertheless, the scientific literature lacks a systematic analysis of these findings. Their systematic study would be fundamental because it may allow to identify common microscopic patterns representative of pulmonary vascular damage: useful data for pathologists in clinical and forensic settings. For these reasons, the authors realized a systematic review of the literature in order to identify the aforementioned data, and to propose their systematic analysis in the light of the scientific literature.

Materials and Methods

At first, asystematic review of the scientific literature was conductedin order to identify articles containing pulmonary histologicaldata of deceased people with positivity for Sars-CoV-2 infection. The

research was performed on Medline electronic database (until November 10th, 2020) using the following algorithm: [histology AND (Sars-Cov-2 OR Covid19)]. The following inclusion criteria were used:articles written in English;articles containing the report of one or more cases in which the deceased had ante-mortem/ post-mortem positivity for Sars-CoV-2 infection;articles reporting specific pulmonary histologicdata for each case.On the contrary, articles containing generic summaries/descriptions of microscopic evidences of more cases were excluded.

The research yielded 2,152 potentially relevant articles. Among them, 33 articles and 3 cross-referencesmatched the aforementioned criteria(Table 1)[3,6-40]. Then, the36articles were analysed in order toidentify onlythe cases in which specific microscopic findings of vascular structures were described. This analysis yielded 23 articlesthat underwent full review (Table 1)[3,6-8,10,15-20,22,26,27,30,31,34-40]. Collected data of the latter articles are available in Table 2 - 4.They were finally reviewed in the light of the scientific literature. Excel statistic formulas were used to calculate Average, Standard Deviation, Median, and Mode.

Table 1: Number of Articles Included/Excluded for pulmonary microscopic findings

Exclusion/Inclusion	Number ofarticles
Excluded reading the title	1622
Excluded reading the abstract	207
Excluded reading the entire article	265
Not in English	25
Positive for pulmonary microscopic findings	33
Cross references positive for pulmonary microscopic findings	3
Reviewed articles (positive for microscopic vascular findings)	23

Table 2: Summary of the most common comorbidities

Comorbidity	Number of cases
Diabetes	42
Heart failure	36
Obesity	17
Aorticstenosis	15
Coronaryarterydisease	8
Kidneyfailure	8
Hypertension	7
Atrialfibrillation	6
Hyperlipidemia	6
Chronicobstructivepulmonarydisease	5
Dyslipidemia	4
Cardiovasculardisease	3
Ischemiccardiomyopathy	3
Obstructivesleep apnea syndrome	3
Congestive heartfailure	2
Dementia	2

Table 3: Quali/quantitative representation of vascular microscopic findings

Vascularlocalization	Findings	Number of cases
Peri-vascularfindings	LC infiltrate Genericinflammatory infiltrate Fibrin	5 (1/5 CD3+) 4 2
Vascularfindings in vessels	Generic thrombi Fibrin thrombi Platelet thrombi Hyaline thrombi Congestion Inflammatory infiltration Fibrin Megakaryocytes Platelets and NET Platelets Complement TE signs	37 (25/37 micro-thrombi) 7 3 (1/3 CD61+) 2 28 6 3 3 3 (PF4+ and H31+) 1 (CD61+) 2 (1/2 5b-9+; 1/2 C4d+,C3d+,C5b-9+) 3
Vascularwalls	Hyperplasia Endotheliitis Necrosis Apoptotic bodies associated with the endothelium	5 5 (3/5 aspecific; 1/5 lymphocytic; 1/5 neutrophilic) 2 1
Neovascularization	Neovascularization	1

LC: lymphocyte, NET: neutrophil extracellular trap, TE: thromboembolism

Table 4: Vessels' caliber in case of thrombi

	Caliber	Number of cases
Caliber of vessels characterized by thrombi	Large Medium Small/capillaries Not specified	1 13 6 19

Results

The 36 articles contained the description of 142 cases. Among them, in 79/142 cases specific microscopic findings of vascular structures were described. Main characteristics of these cases are summarized in Table 2 - 4. Average age of the 79 cases was 69.64 years old (Standard Deviation 13.81, Median 73, Mode 71). Their sex distribution was:male 52, female 23, 4 not specified. The most common comorbidities werediabetes (42), hypertension (36 cases), and obesity (17 cases).Quali/

quantitative analysis of vascular microscopic findings issummarized in Table 3and4.

Discussion

In the literature, multiple reports of microscopic pulmonary findings of vascular structures in patients deceased by or with SARS-Cov-2 infection are available[1-4]. However, these reports usually refer to single cases or small case series[1,3]. Thus, until now the scientific literature lacks a systematic analysis of these findings. In particular,common microscopic patterns of pulmonary vascular involvement/damage are not described. The present review pointed out the following indications on this topic.

COVID19 and coagulative thromboticmicroscopic patterns

The results of the present review did not allow to identify a vascular pattern that was present in all reviewed cases. However, common findings were intravascular thrombi that recurred 49 times. The most part of themwere described as micro-thrombi and/or identified in medium/small/capillary vessels. At microscopic evaluation, large vessel involvement was reported only in one case. In the scientific literature,pulmonary microvascular thrombosis was early suggested as responsible for COVID19 progression[41,42], observing that infected patients were characterized by "profound hypoxia which was out of proportion to the preserved lung mechanics suggestive of significantpulmonary shunting, raising the possibility of a lung injury mechanism different from that oftraditional ARDS"[2].This statement was confirmed by Ackerman and colleagues who compared lungs of influenza Ainfectedpatients against SARS-CoV-2 ones, revealing as micro-thrombi were more prevalent in COVID-19[1]. It is well known that viral infections – via multiple pathways – can impair the coagulation cascade causing haemorrhagic and/or thrombotic complications[2]. Indeed, in COVID19 one of the most common manifestations of altered coagulation cascade is the elevation of D-Dimer that is"a marker of coagulationcascade activation in the microvascular beds which has been shown to be pathologically elevated in 46%of SARS-CoV-2-infected patients and in 56% of those with severe disease"[2]. Typical features of coagulopathy in SARS-CoV-2 infected patients (i.e.D-Dimer, fibrinogen, von Willebrand factor, and VIII factor elevation, mostly normal or slightly depressedpartial thromboplastin time(PTT), variable variation of platelet count, and normal XIa factor)suggest the prevalent activation of extrinsic coagulation cascade[2]. The latter is also known as the tissue factor pathway because it is triggered by tissue trauma and endothelial activation. This event causes the expression of high levels of tissue factor on vascular cells, resulting in activation of coagulation factors[2].Several authorssuggested direct and/or indirect SARS-CoV-2 ability to cause vascular tissue trauma/endothelial activation[2,3,43]. The direct activation hypothesis is based on SARS-CoV-2 ability to infect the host through the angiotensin converting enzyme 2(ACE2) receptor which is also expressed by endothelial cells[3]. On the contrary, the indirect activation one has foundation in the so-calledcytokine storm (principally IL-1 and IL-6 elevation) caused by the virus[2].

In addition, it is important to note that in two cases the authors reported vascular deposits of C5b-9at immunocytochemistry, demonstrating complement activation (especially the alternative pathway). The latter finding is particularly suggestive because it is well known that complement system cross talks with the coagulation cascade at different levels[2,44,45]. In particular, the complement can induce tissue factor expression on the endothelium, and it can suppress mast cells' fibrinolytic activity causing clot progression[2,46]. Thus, this phenomenon can be considered as a possible cause/concurrent cause of the aforementioned thrombosis in COVID19. Similar considerations can be related to the so-called neutrophil extracellular traps (NETs) that were identified in three cases at immunohistochemistry.NETs are principally composed by decondensed chromatin (DNA and histones) that is released by neutrophiles to immobilize microorganisms when they are activated by strong stimulations[43]. Recent studies highlighted NET's immune-thrombosis ability in COVID19, demonstrating that "NET release is positively correlated with invivo

thrombotic potency in COVID-19"[47]. In particular, these complexes would be capable to activate tissue factor/thrombinaxisand platelets[43,47].Indeed, different authors recognized NETs as linking factors between inflammation, coagulation, and thrombosis (locally and systemically)[43,48].In addition, from a clinical point of view the aforementioned data are particularly meaningful because they seem to agree with the recent manuscript byZuo and colleagues whoreported the correlation between cell-free-DNA levels (NETosis) and acuity of COVID19, inflammatory response, and need for mechanical ventilation[49].

Talking about megakaryocytes, multiple studies suggested their common presence inSARS-CoV-2 affected lungs[50]. In the present review, megakaryocytes'population was identified in vessels in three cases. Despite this small number, the latter microscopic finding seems to agree with the scientific literature that highlightedmegakaryocytes'altered functions in SARS-CoV-2 disease. In particular, Bernardes and colleagues recently "hypothesized that altered presence and function of MKsmight be a distinct feature of COVID-19", describing higher number of megakaryocytes in severe COVID19 and reporting "an increased metabolic activityof MKs along the disease trajectory compared with that inhealthy controls"[50]. Thus, megakaryocytes may have a specific role in SARS-CoV-2 infection, however neither literature data nor the present review pointed out a common vascular microscopic pulmonary pattern of these cells.

In the light of the above, the aforementioned results allowed to identify vascular thrombosis (especially in lesser caliber vessels) as common microscopic pattern of pulmonary parenchyma in SARS-CoV-2 infected patients in lethal cases. The recurrence of this pattern is confirmed by scientific literature data which demonstrate SARS-CoV-2 ability to interfere with coagulation cascade. However, so farSARS-CoV-2 pro-coagulant mechanisms are not completely understood. In addition, it is not yet defined the reasons why thrombosis activation tends to involve especially lessercaliber vessels.

COVID19 and vascular/endothelialinflammatory patterns

Lymphocytic endotheliitis and apoptotic bodies are already described in surgical tissue specimens of SARS-CoV-2 infected patients. These findings are considered as the result viral interaction with endothelial cells. Even if SARS-CoV-2 ability to infect engineered human blood vessels – binding ACE 2 receptors of endothelial cells – is already demonstrated[51], it is important to highlight that apoptosis may not be triggered only by viral entry. The binding with endothelial cells' surface can activate apoptotic pathway signaling[52]. In the present review, apoptotic bodies within the endothelium were described in one case. Thus, the latter finding cannot be identified as a common vascular microscopic pattern, even if literature data report direct and/orindirect viral activityon the endothelium. Similar considerations can be related to endotheliitis that was reported in 5 cases. In particular, in 2/5 cases the authors respectively described this inflammatory phenomenonas lymphocytic and neutrophilic.Immunology of COVID19 is not yet completely understood[5], even if preliminary data suggest the following considerations on lymphocytic and neutrophilic cells:as mentioned above, neutrophilic cells seem to be implicated in NET formations; several reports pointed out the occurrenceof T lymphopenia (reduction of $CD4^+$ and $CD8^+$counts in peripheral blood in moderate and severe COVID19); on the contrary, the scientific literature highlighted that SARS-CoV-2 elicits a significant B cell response, determining rapid increase of virus-specific IgM, IgG and IgA, and neutralizing IgG antibodies[5]. Thus, the localization of lymphocytes and neutrophilesin correspondence with the endothelium is consistent with literature data, even if – especially for lymphocytes – in the reviewed articles there were few immunohistochemistry studies which allowed to identify cells'specific sub-types.In the present review, 15 cases were characterized by vascular/peri-vascular inflammatory infiltration. Among them in 5/15 cases the authors specifically described lymphocytes as prevalent population; on the contrary, in 10/15 cases they did not described cells'specific pattern. In addition, in

only 1/15 case at immunohistochemistry lymphocytes were characterized as $CD3^+$. Thus, even if these data are consistent with literature's indications, it was not possible to identify a specific vascular/peri-vascular microscopic pattern.

In the light of the above, the aforementioned results allow to state that – as for COVID19 immunology – until nowcommoninflammatory microscopic patterns of endothelial/vascular pulmonary structures are not clearly identified in SARS-CoV-2 infected patients in lethal cases.

COVID19 and pulmonary thromboembolism

In the scientific literature, pulmonary thromboembolism (PTE) was reported as a common finding in patients admitted to intensive care units. For example, in a cohort study of 107 patients the 20% was affected by PTE despite thromboprophylaxis[53]. The comparison of this cohort "to a similar one hospitalized a year earlier and a smaller influenza cohort from the prior season" yielded the following result: "the frequency of PE was twice as high in the COVID group despite less computed tomography (CT)angiogram tests performed"[53]. In the present review, even if multiple findings of thrombosis (especially micro-thrombosis) were identified, specific signs of PTE were described in 3 cases. Thus, the present review did not allow to define microscopic PTE signs as common in lung parenchyma of SARS-CoV-2 deceased patients.

COVID19 and neovascularization

Pulmonary microscopic neovascularization was reported in one case. Thus, this finding cannot be considered common of SARS-CoV-2 infection in deceased individuals. However, Ackerman and colleagues recently pointed out the recurrence – at transmission electron microscopy – of new blood vessel formation by enhanced intussusceptive angiogenesis in lungs of patients who died from COVID19[1]. This type of angiogenesis does not occur by conventional sprouting of new vessels, but it is characterized by "thepresence of a pillar or post spanning the lumenof the vessel"[1]. In addition, the authors reported that vascular angiogenesis allowed to distinguish "the pulmonary pathobiologyof Covid-19 from that of equally severe influenza virus infection"[1], suggesting that "althoughtissue hypoxia was probably a common featurein the lungs from both these groups of patients,we speculate that the greater degree of endothelialitisand thrombosis in the lungs from patientswith Covid-19 may contribute to the relativefrequency of sprouting and intussusceptiveangiogenesis observed in these patients"[1]. Even if further studies are necessary in order to clearly understand this phenomenon (especially its relations with the clinical course of the disease), the abovementioned statement is confirmed by the high number of cases in which vascular thrombosis and/or endotheliitis were described in the present review.

COVID19 and vascular wall hyperplasia

Significantgrowth ofvascular endothelial and myointimalcellswasreportedinskinbiopsiesofcutaneous lesions during SARS-CoV-2infection period[54]. The authors reported a skinvasculopathic reaction pattern, suggesting "amicrovascular process with vascular wall cell injury"and vascularcell proliferation "confirmed by the increased numbers ofreplicating cells positive stained for Ki67 and Cyclin D1 found inboth vascular endothelial cells and myointimal vascular cells"[54].In addition, they pointed out that the possible underlying pathophysiological process would rely on vascular response to hypoxic insult[54,55], which is considered as a fundamental feature of COVID19[2]. The present review identified vascular wall hyperplasia of pulmonary parenchyma in 5 cases.However, these cases – especially in relation to their low number – did not allow to identify specific microscopic pattern which could be useful to understand the underlying pathophysiological process or to define these findings as common in in SARS-CoV-2 infected patients in lethal cases. Further studies will be necessary on this topic.

Conclusions

This review representsthe first systematic analysis of microscopic pulmonary findings of vascular structures

in patients deceased by or with SARS-Cov-2 infection. It allowed to identify vascular thrombosis (especially in lesser caliber vessels) as common microscopic pattern of pulmonary parenchymain these patients. The recurrence of this pattern is confirmed by scientific literature data which demonstrate SARS-CoV-2 ability to interfere with the coagulation cascade. However, until now SARS-CoV-2 pro-coagulant mechanisms are not completely understood. In addition, it is not yet defined the reason why thrombosis activation tends to involve especially less caliber vessels.

This analysis also pointed out the recurrence of other meaningful pulmonary microscopic findings of vascular structures. So far, they cannot be defined as common in SARS-CoV-2 infected patients in lethal cases; indeed, further studies will be necessary to reach this goal.

Limitations of the present review are related to the non-homogeneityof microscopic evaluations of histologic slides. In particular, the aforementioned results came from operators who have different backgrounds and different ways to describe microscopic pulmonary findings. Indeed, in the present review the most difficult task relied on interpretation and aggregation of the same/similar microscopic findings in quali/quantitative groups that would have allowed to implement a coherent systematic analysis avoiding results' distortions. For this reason, the authors divided microscopic findings in few and simple categories (peri-vascular findings, vascular findings in vessels, vascular walls, and neovascularization) in order to reach this goal.

Another limitation was related to the impossibility to clearly distinguish between patients deceased with or by SARS-CoV-2 infection.Thus, all indications of the present review are referred to SARS-CoV-2 infected patients in lethal cases. In addition, it cannot be excluded that some microscopic results could be the manifestation of other comorbidities and/or pathophysiologic processes. For this reason, the authors also proposed a systematic analysis of results in the light of the scientific literature, in order to verify their coherency with the available literature data.

Acknowledgements: Nil.

Ethical Clearance: Taken from University of Turin ethical committee.

Source of funding: Self-Funding.

Conflict of Interest: Nil

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