**PATHOLOGICA 2023;**115:275-283;
DOI: 10.32074/1591-951X-906

Review

# Exploring the pathologist's role in understanding COVID-19: from pneumonia to long-COVID lung sequelae

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**Summary**

The crucial role of pathologists in enhancing our understanding of SARS-CoV-2-related disease, from initial pneumonia manifestations to persistent long COVID lung symptoms, is the focus of this review. Pathological explorations have offered unprecedented insights into the early stages of severe COVID-19, shedding light on the interplay between the virus and subsequent complications, thereby shaping clinical approaches. Growing interest is directed to residual lung abnormalities of COVID-19 survivors. Although various radiological studies reported long-lasting pulmonary changes (e.g., ground glass opacities, reticulations, and bronchiectasis), the true incidence of pulmonary fibrosis and corresponding pathological findings in these patients remains largely unknown. There are a few high-impact and knowledgeable works on late complications in COVID-19 survivors, several coming from explant or autopsy cases, and rare cases from in vivo sampling. The study of biopsy samples has further deepened our knowledge of the aftermath of COVID-19 on lung tissue, uncovering alterations at the cellular level and shifts in vascular and epithelial dynamics. Despite the substantial progress made, future research is needed to devise a uniform strategy for interpreting lung biopsies, with a focus on leveraging advanced tools such as molecular and digital pathology techniques, along with artificial intelligence.

**Key words:** SARS-CoV-2, pathology, pulmonary sequelae, review, long COVID-19

Received and accepted: July 21, 2023

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**How to cite this article:** Olteanu GE, Pezzuto F, Lunardi F, et al. Exploring the pathologist's role in understanding COVID-19: from pneumonia to long-COVID lung sequelae. Pathologica 2023;115:275-283. https://doi.org/10.32074/1591-951X-906



## Introduction

Since COVID-19 pneumonia was first reported, the contribution of pathologists during various pandemic waves has been instrumental in understanding the pathophysiology of SARS-CoV-2-related disease. This review discusses in the first part the role of pathologists in studying the 'early lesions' of COVID-19 pneumonia, while the subsequent section focuses on long COVID-19 lung lesions, citing evidence from high-impact, peer-reviewed, and indexed scientific journals.

For the second part concerning long COVID-19 lung lesions, we conducted a Medline-indexed study using PubMed as the primary database, applying different search strings on July 1, 2023. The objective was to include only those articles where pathological studies – autopsy, explant, biopsy, and ancillary methods – were used to assess long COVID-19 lung sequelae. A search for 'long COVID-19' combined with 'autopsy', 'explant', 'biopsy', 'histology', 'pathology', 'immunohistochemistry', and 'molecular investigations' yielded 14 articles relevant to the aim of our study.

## COVID-19 pneumonia - key lessons learned from early lesions

**During the first wave,** knowledge about the pathobiology of SARS-CoV-2 infection in the lung was mainly acquired from autopsy studies. COVID-19, caused by the SARS-CoV-2 virus, profoundly impacts the respiratory system. The pathological features of COVID-19 are distinct and multifaceted, involving alveolar damage, vascular injury, and airway damage [1-5].

### Alveolar damage

The primary target of SARS-CoV-2 in the lungs is the alveolus, the small air sacs responsible for gas exchange. Histology of autoptic cases showed diffuse alveolar damage (DAD) features with pneumocyte type 2 hyperplasia, and linear interalveolar fibrin deposition [4,5]. In some cases, these changes are focal, with only mild interstitial edema. However, other cases present homogeneous fibrin deposits and marked interstitial oedema with early interalveolar organization [6].

### Vascular injury

Vascular injury is a frequent lesion in COVID-19 pneumonia, either in pure form or in combination with alveolar injury [7]. The pulmonary vasculature consistently show thrombi in pulmonary arteries with a diameter of 1 to 2 mm, without complete luminal obstruction [6]. Alveolar capillary microthrombi are significantly more prevalent in patients with COVID-19 than in patients with other viral pneumonia, such as influenza [5,6]. The lungs of COVID-19 patients also exhibit distorted vascularity with structurally deformed capillaries, showing sudden changes in calibre and the presence of intussusceptive pillars within the capillaries. Ultrastructural damage to the endothelium is evident, as well as the presence of intracellular SARS-CoV-2 [6].

### Airway damage

Airway damage in COVID-19 is less well defined but is inferred from the extensive damage to the alveoli and vasculature [7]. The larger airways – including the trachea and larger bronchi – were almost clear of mucus plugs, and evidence of mucosal ulceration was present in the form of discrete white patches. These patches were found to contain a diverse mix of inflammatory cells. Despite the general patency of the airways, a substantial portion of patients demonstrated considerable inflammation within the large airways. This inflammation manifested in both acute and chronic forms. Furthermore, these findings did not show any significant correlation with long periods of intubation or bacterial or fungal pneumonia, suggesting a more direct SARS-CoV-2-directed injury [5,7].

### Acute Respiratory Distress Syndrome (ARDS) and COVID-19

The severe respiratory complication associated with COVID-19, named acute respiratory distress syndrome (ARDS), is fundamentally driven by a complex clinical picture and heterogeneous pathological lesions, both involving alveolar and vascular components [8-10]. Pathological studies analyzing several lesions in autoptic cases using a machine learning approach identified a mixed phenotype in patients characterized by vascular lesions and alveolar damage. The vascular injury phenotype was consistently present in most cases either as a pure form or in combination with alveolar injury. Phenotypes with more severe alveolar injury showed significantly more frequent tracheal intubation, longer duration of invasive mechanical ventilation, illness, intensive care unit and hospital stay, and lower tissue viral quantity. Furthermore, in this phenotype, superimposed infections, tumors, and aspiration pneumonia were also more frequent [11].

All these important findings concerning complex inflammatory processes, sporadically present virally infected cells, and low copy number of SARS-CoV-2 supported the use of immunomodulatory therapies such as low-dose dexamethasone in the treatment of severe COVID-19 [10].

**The second wave of COVID-19,** based on the experience of European pulmonary pathologists throughout the first two waves of the pandemic, has shed further light on the pulmonary pathology associated with COVID-19. Fortarezza et al. provided a survey study based on expert pulmonary pathologists with a comparative analysis of the pathological changes observed in the lungs of patients who succumbed to COVID-19 during the first and second wave of the pandemic [11].

In the study, they found several key differences between the two waves. During the second wave, there was a marked increase in the frequency of pulmonary superinfections, both bacterial and particularly fungal, compared to the first wave. Notably, the incidence of COVID-19-associated pulmonary aspergillosis (CAPA), a fungal co-infection, rose significantly during the second wave [11].

CAPA refers to the co-infection of the respiratory tract by the *Aspergillus spp.* species in patients with COVID-19, particularly those with severe disease and prolonged hospitalization [12]. These cases exhibit complex alterations in the lung microenvironment, involving the coexistence of SARS-CoV-2 infection with other respiratory pathogens, such as bacteria and fungi. Comprehensive bronchoalveolar lavage (BAL) characterization has provided valuable insights into the cellular components, inflammatory signature, and respiratory

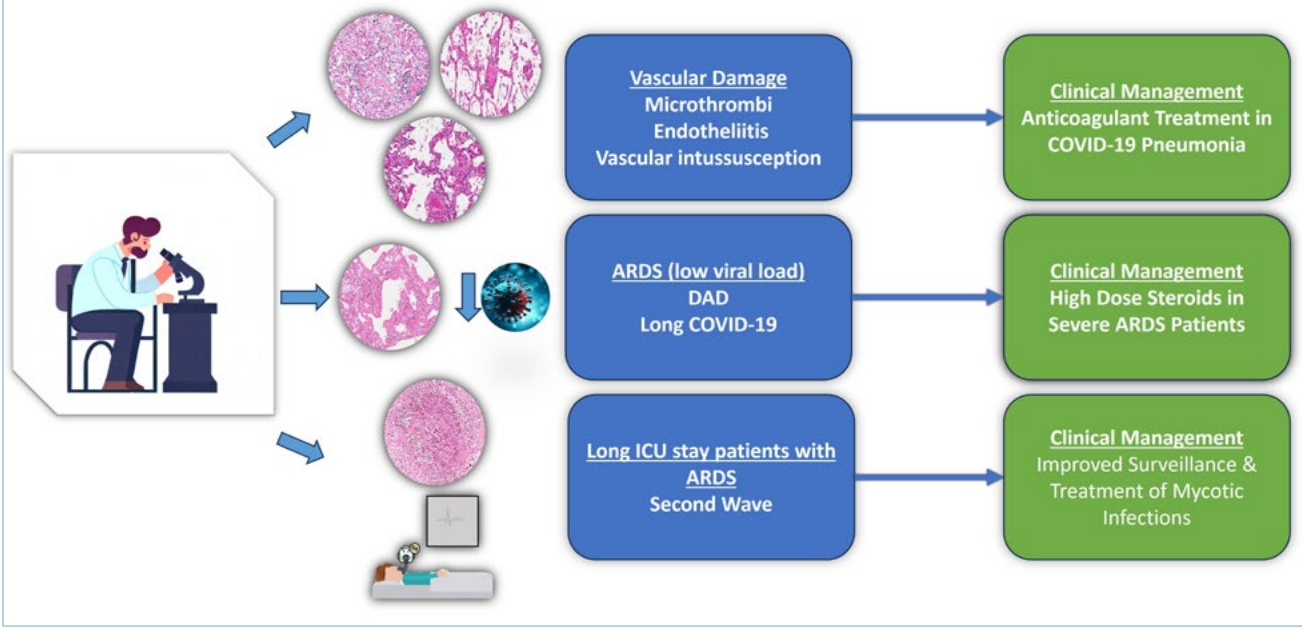


**Figure 1.** This illustration demonstrates the pathologist's contribution to understanding the pathophysiology of SARS-CoV-2 infection and COVID-19 pneumonia, as well as the progression of clinical management that stemmed from these insights.

pathogens associated with CAPA. Notably, the presence of SARS-CoV-2, along with other respiratory pathogens, was detected in BAL samples, emphasizing the complexity of the lung microenvironment in CAPA patients [9].

Since the discovery of these complications in 2021, the European Confederation of Medical Mycology (ECMM) and the International Society for Human-Animal Mycology (ISHAM) established consensus criteria to help diagnose and manage CAPA [13]. Several centers have implemented diagnostic approaches even with the use of invasive procedures i.e., transbronchial biopsies or less invasive procedures i.e., BAL to detect this kind of infection.

The impact of pathological studies on our understanding of lesions of severe COVID-19 pneumonia can be succinctly summarized as having produced progress in our knowledge of the pathophysiology of viral damage and virus-associated complications. This progress has yielded vital insights for clinical management (Fig. 1).

## Pioneering insights: integrating autopsy and transplant findings (2020-2021)

In the quest to understand the long-term impacts of COVID-19, autopsy and transplant studies have provided invaluable insights. Both these research pathways have not only been pursued independently but have often intersected, enhancing our understanding of the disease and its effects. The following chronological summary of key studies involving both of these pathways highlights the significant findings and their implications.

A ground-breaking study by Bharat et al. in 2020 set the stage for subsequent research combining the autopsy and transplant pathways [14]. The team conducted lung transplantations on three patients with severe, non-resolving COVID-19-associated ARDS. The first patient underwent a lung transplant approximately 9 weeks after the initial disease onset, the second patient underwent a lung transplant after 14.7 weeks, and the third patient underwent a lung transplant 12.8 weeks after developing ARDS. In the study, histological analyses of lung tissues from the three severe COVID-19 patients who underwent lung transplantation revealed shared characteristics [14]. The explant lungs were notably heavy and showed dense pleural adhesions, cavities with necrosis, and bacterial pathogens on analysis. The microscopic investigation further displayed extensive interstitial thickening, fibrosis, bronchiolitis, bronchiolar fibrosis, honeycombing changes, alveolar hemorrhage and evidence of iron deposition observed in alveolar macrophages. Notably, multiple cystic structures in varying stages of formation were identified, possibly due to prolonged mechanical ventilation or resulting from viral or bac-

terial infection [14]. The authors concluded that the severe lung damage in these patients was irreversible and that transplantation was the only viable treatment option. Moreover, the study found that bronchoscopic sampling could be a clinically useful method to exclude SARS-CoV-2 infection before consideration for a lung transplant. Additionally, the authors did not detect the SARS-CoV-2 viral genome in the explant lungs. The authors also suggested a model for pulmonary fibrosis in which injury, often virally mediated, triggers the formation of reciprocal circuits between macrophages and fibroblasts, leading to fibrotic lung disease [14]. The authors proposed bilateral rather than single lung transplantation for severe COVID-19 patients, after considering transplantation only when a possible spontaneous lung recovery was excluded, and after involving patients in the transplant decision process whenever possible [14].

In 2021 Aesif et al. reported the antemortem pathologic findings in the lungs of two patients who survived severe COVID-19 for extended periods but eventually succumbed to the disease, and also reported the pathological findings in explant lungs of a patient who underwent a lung transplant after prolonged severe COVID-19 [15]. Patient number 1 was a 46-year-old Black man with hypertension, obesity, and chronic lymphocytic leukemia who presented with moderate respiratory symptoms due to COVID-19. He was intubated on day 18. On day 21, he was started on venovenous extracorporeal membrane oxygenation (V-V ECMO) due to persistent hypoxemia. A series of complications, including thromboses, bleeding, pneumothorax, hemothorax, and lung parenchymal-pleural fistula, persisted throughout his treatment. His health continued to decline and he died on day 57. Histological examination of the debrided right middle lung lobe confirmed extensive infarct-like necrosis of the lung with “ghosts” of alveolar septa lined by hyaline membranes. Extensive colonization of necrotic lung by budding yeast (Candida albicans) was also observed. Overall, the pattern of lung injury was compatible with DAD in the acute stage. No significant fibrosis was observed. Patient number 2 was a 57-year-old obese Hispanic woman with a distant myocardial infarction who presented with respiratory symptoms due to COVID-19. Despite treatment, her respiratory status worsened and she was put on ECMO on day 12. She died on day 74. Histologic examination showed diffuse interstitial expansion, vaguely reminiscent of non-specific interstitial pneumonia (NSIP). Focal microscopic honeycomb change was also observed, alongside patchy interstitial lymphocytic infiltrates, and mild chronic pleuritis with fibrinous exudates. Most of the interstitial fibrosis observed was mature collagen-type, and no definitive hyaline membranes, Masson bodies, or fibroblastic foci were appreciated. No capillaritis/vasculitis or thromboembolic changes were observed. Patient number three was a 57-year-old man with coronary artery disease and hypertension who was diagnosed with COVID-19 and subsequently developed progressively worsening hypoxic respiratory failure. He was put on mechanical ventilation on day 14 and on V-V ECMO on day 54 due to worsening pulmonary parameters. Despite treatment and multiple negative molecular tests for SARS-CoV-2, his condition did not improve. On day 126, he underwent bilateral sequential lung transplantation. Histopathologic examination of the explanted lungs revealed mild diffuse interstitial chronic inflammation with diffuse, relatively uniform-appearing interstitial expansion, vaguely resembling NSIP. Peribronchiolar metaplasia was also present but not extensive. Foci of microscopic honeycomb change were also present. No evidence of acute lung injury or capillaritis/vasculitis was found. Most of the interstitial expansion was composed of collagen-type fibrosis [15]. The authors concluded that following recovery from COVID-19 and prolonged hospitalization, there can be the development of diffuse interstitial fibrosis with early microscopic honeycomb change [15].

Later in the same year, Bharat et al. published a case series focusing on lung transplants for patients with severe COVID-19-associated ARDS in the USA, Italy, Austria, and India [16]. From May to September 2020, 12 of these patients underwent bilateral lung transplantation at six leading transplant centers in these countries. The patients, predominantly male with a median age of 48, all showed severe lung damage that did not improve despite extensive support, including mechanical ventilation and extracorporeal membrane oxygenation [16].

The pathological examination of explanted lungs from all patients revealed extensive damage, DAD, involving large areas of the lung parenchyma. The length of time from the onset of COVID-19 to transplantation, which ranged from 40 to 118 days, was positively associated with the prominence of pulmonary fibrosis. Other significant findings included cavities with necrosis, areas of bronchopneumonia resulting from secondary bacterial infection, and acute interstitial pneumonitis. This pneumonitis was characterized by acute neutrophilic infiltrates within the interstitium and alveolar spaces. Other observed features were interstitial expansion due to fibrosis, bronchiolisation of alveoli, and microscopic honeycombing. In some patients, thrombi were observed in small and intermediate vessels, with or without recanalization. Furthermore, the use of SHIELD tissue-clearing technology to evaluate explanted lung tissue revealed architectural distortion

akin to that observed in other end-stage lung diseases such as emphysema and α1-antitrypsin deficiency. For individual patients, the time from the initiation of mechanical ventilation to lung transplantation ranged from 39 to 114 days [16].

This study, building on their previous work, indicated that lung transplantation could be a viable treatment option for selected patients with severe, non-resolving COVID-19-associated ARDS [16].

In 2022, a comprehensive study by Roden et al. analyzed various types of tissue samples, including autopsies, from patients with COVID-19 whose onset of symptoms or confirmed diagnosis was more than 28 days before the procedure [17]. The study suggested that acute lung injury (ALI) and SARS-CoV-2 RNA can persist in patients with COVID-19 for many months, and ALI may progress to fibrotic interstitial lung disease. This information was crucial for our understanding of the long-term impacts of COVID-19 and for guiding transplant decisions [17].

Maccio et al. in 2022 conducted a series of 35 COVID-19 autopsies to investigate the presence of postmortem SARS-CoV-2 RNA and its correlation with morphologic findings [18]. They found that SARS-CoV-2 RNA can persist up to 40 days after the first diagnosis and remain detectable postmortem [18]. Lung tissue exhibited the highest frequencies of SARS-CoV-2 RNA positivity, and late-stage tissue damage was observed in several organs [18].

These findings highlight the persistence of SARS-CoV-2 RNA and the long-term effects of COVID-19. It should be stated that the histological features, for example, fibrosis and ALI, in the lungs of long COVID-19 patients could represent lesions already present before viral infection, or it could be that additional factors like prolonged medical interventions facilitate the occurrence of these pathological features [18].

All autopsy studies provide important insights into the persistence of SARS-CoV-2 RNA, organ-specific effects, and long-term tissue damage associated with COVID-19. Indeed, the authors underlined the presence of acute lesions and the SARS-CoV-2 viral genome in the tissue even far from the acute episode of COVID-19.

However, most of the authors underlined that these persistent symptoms and organ damages are not necessarily directly caused by the virus itself. While the SARS-CoV-2 virus can cause significant damage during the acute phase of the infection, the long-term effects seen in post-COVID and/or long-COVID syndrome may also be due to the body's immune response to the virus, other individual health factors, or a combination of these elements. Therefore, while the virus is a key factor, it is not the sole determinant of these persistent symptoms and damages. This understanding is essential for a comprehensive approach to managing and treating these long-term effects.

## Understanding post-COVID and long-COVID through biopsy-centered studies

Biopsy studies have provided detailed insights into the pathological changes occurring in the lungs of individuals recovering from COVID-19. These insights have shed light on post-COVID-19 lung disease. Although in vivo or biopsy studies are less common compared to autopsy or explant studies, they have been crucial. This is because, unlike autopsy (autoptic) and explant studies, biopsy studies typically lack the confounding factors of agonal lesions (those that occur at the moment of death) and ischemic lesions (typically seen in explant studies). Here, we elaborate on the findings from each of the biopsy studies, highlighting their contributions to our understanding of post-COVID-19 lung pathology.

Pogatchnik et al. explored the case of a 61-year-old woman, previously in good health, who, despite having a negative nasopharyngeal RT-PCR test for SARS-CoV-2, exhibited symptoms consistent with COVID-19 for three weeks [19]. Five days post-admission, transbronchial biopsies taken from various peripheral regions of the right lung revealed scattered fibromyxoid plugs within distal airspaces, further supporting the OP (organizing pneumonia) pattern. Subsequently, RT-PCR for SARS-CoV-2 returned a positive result from a BAL; other BAL cultures were unremarkable [19]. CT imaging conducted 26 days post-presentation showed minimal residual opacities [19].

This case study underscores the potential for lung tissue damage and alterations even in instances of negative PCR tests. The findings confirmed both radiographically and histologically, suggest that OP viewed as an active and sometimes aberrant lung repair process, may depict the evolution of COVID-19 in patients with mild to moderate disease [19].

Doglioni et al. conducted a study involving transbronchial lung cryo-biopsies performed on 12 COVID-19 patients within 20 days of symptom onset [20]. The histological evaluation of the pulmonary structure revealed significant modifications, including alveolar architecture distortion, type II pneumocyte hyperplasia (TIIPH), and diffuse vascular modifications. Notably, hyperplasia of TIIPH was observed in 9 out of 12 cases, exhibiting a unique "patchy" distribution. Vascular changes, such as dilated and hyperplastic interstitial capillaries and venules, were present in all cases. A patchy lymphocyte infiltrate was observed in 9 out of

12 cases, while interstitial plasma cells, neutrophils, and eosinophils were either absent or extremely rare. Irregular clusters of mononuclear cells were found within alveolar spaces in 9 out of 12 cases.

*In situ,* analysis and immunohistochemistry revealed the presence of the SARS-CoV-2 viral genome in scattered cells recognized as TIIPH. The proliferation index in the TIIPH sprouts or nodules was over 50%. Strong nuclear expression of phosphorylated STAT3 (pSTAT3) was demonstrated in more than 50% of TIIPH in all of 9 cases investigated. Irregular clusters of mononuclear cells, characterized as macrophages, were present within alveolar spaces in 10 of 12 cases. These cells exhibited an unusual "hybrid" phenotype including dendritic-cell markers. The blood vessels showed an increased number of capillaries in the alveolar septa, and endothelial cells presented a rather unexpected immunophenotype, which included strong nuclear expression of pSTAT3 and strong diffuse expression of PD-L1 and IDO. The authors highlighted the heterogeneity of lung damage in COVID-19 patients parallel to those seen in autopsy studies [20].

Konopka et al. performed a retrospective observational study where they examined surgical lung biopsies (SLB) from patients with persistent interstitial lung disease (ILD) following recovery from acute COVID-19 [21]. The most common histopathologic diagnosis observed was usual interstitial pneumonia (UIP), demonstrating a potential long-term complication of COVID-19. However, the authors noted that these findings were predominantly present in older patients with a history of chronic lung disease prior to SARS-CoV-2 infection, and thus at least some UIP lesions could have occurred before COVID-19 pneumonia. However, the authors questioned the real link of the UIP pattern as a consequence of SARS-CoV-2 infection. Indeed, the authors noted that these lesions were more prevalent in older patients with a history of chronic disease and that the UIP pattern could have been from a pre-existing disease more than a sequela of COVID-19 [21].

Roden et al. collected various types of tissue samples (e.g., from autopsies, explants, surgical lung biopsies, transbronchial biopsies, cryo-biopsies, and needle biopsies) from patients with COVID-19 whose onset of symptoms or confirmed diagnosis was more than 28 days before the procedure [17]. Histologically, the lung tissues from biopsies i.e., excluding autopsies and explants, showed DAD with hyaline membrane formation, commonly found in ARDS [17]. Additionally, there were instances of OP and vascular changes, including thrombi and angiogenesis. Some patients displayed fibrosis, suggesting a progression towards a chronic disease state. The study also found features of ALI in the majority of specimens, even up to 298 days post COVID-19 onset, suggesting that ALI and SARS-CoV-2 RNA can be detected in patients with COVID-19 for many months, and ALI may progress to fibrotic ILD [17].

In a case series, Baldi et al. explored the potential of transbronchial biopsies as a tool to evaluate patients with long COVID-19 and pulmonary involvement [22]. The investigation was conducted during the late stage of pulmonary involvement, specifically 4-15 months post-acute infection. Their consistent histopathological findings revealed the presence of bronchiolocentric interstitial pneumonia, with the majority of the cases presenting architectural distortion and peribronchial remodelling accompanied by extracellular matrix deposition. These results signal the potential utility of lung biopsy in monitoring individuals who show persistent respiratory symptoms and long-term lung abnormalities following COVID-19.

Despite these promising findings, the authors concluded that the specific indications, appropriate procedures, and optimal timing for lung biopsies in the setting of long COVID-19 warrant further investigation. They proposed that transbronchial biopsy might serve as a primary step in the assessment of patients with post-COVID-19 pulmonary fibrosis, particularly in the context of ongoing symptoms, lung function impairment, and persistent interstitial lung abnormalities on CT scans.

The authors advocate for additional studies to charac-

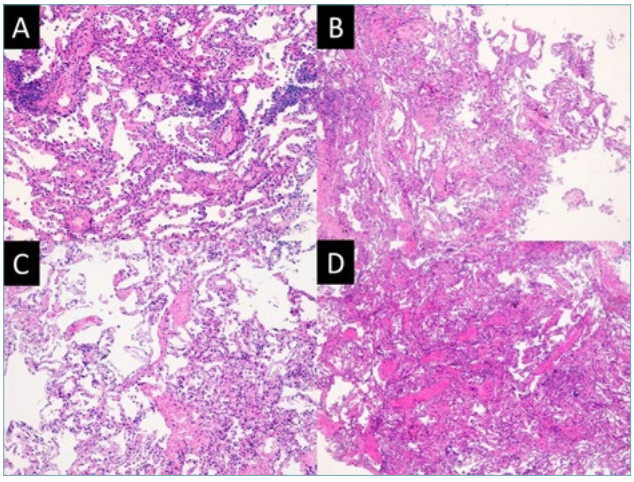


**Figure 2.** This panel illustrates four representative histopathological features of cluster two from biopsies of two different patients (hematoxylin & eosin (H&E) stain all images and all images are at 10x magnification). A) and B) classic organizing pneumonia with lymphocytic interstitial infiltration. C) and D) cicatricial OP.

terize the histopathological patterns in a larger cohort of lung tissue samples obtained from either transbronchial cryo-biopsy or surgical biopsy. Moreover, they emphasize the need to explore the indications for and responses to pharmaceutical treatments, including corticosteroids and antifibrotic drugs, in such settings [22].

Ravaglia et al. conducted a study investigating patients with persistent lung disease following SARS-CoV-2 infection [23]. For the first time, the authors used transbronchial lung cryo-biopsy, identifying three distinct histopathological clusters associated with post-COVID-19 lung disease. The study involved 164 patients, of which 10 patients with parenchymal lung disease extent > 5% underwent cryo-biopsy. The histological pattern was not homogeneous, and three different case clusters were identified [23].

Cluster one, termed "chronic fibrosing," was characterized by post-infection progression of pre-existing interstitial pneumonia. Cluster two (identified five patients), "acute/subacute injury," was characterized by different lung injury types and grades, ranging from OP and fibrosing NSIP to DAD (Fig. 2). Cluster three, "vascular changes," was characterized by diffuse vascular increase, dilatation, and distortion (capillaries and venules) within otherwise normal parenchyma.

In clusters two and three, immunophenotypical changes similar to those observed in early/mild COVID-19 cases of pneumonia were noted, such as abnormal expression of STAT3 in hyperplastic pneumocytes and PD-L1, IDO, and STAT3 in endothelial cells. This study was the first to correlate histological/immunohistochemical patterns with clinical and radiological pictures of patients with post-COVID lung disease [23]. The authors concluded that a comprehensive, multidisciplinary evaluation of these patients is essential to define key patient subgroups and optimize management strategies. This study emphasizes the heterogeneity of post-COVID lung disease and the need for tailored management strategies [23].

Boehm et al. presented a case report describing the potential for pulmonary recovery even in severe cases of COVID-19 infection [24]. The study focused on a 70-year-old man who underwent tumor lobectomy after long-term mechanical ventilation for COVID-19 pneumonia. A histopathological examination of the patient's lung showed no signs of severe damage, but some minor fibrosis possibly linked to the patient's history of smoking. Despite having a severe COVID-19 infection and associated risk factors, including being 70 years old, and despite having arterial hypertension and a history of smoking, the patient regained high respiratory functionality within six months. This recovery contradicts established literature that expects severe long-term respiratory sequelae after a critical COVID-19 infection, particularly in elderly men with arterial hypertension. The case provides hope that good functional status is achievable with optimal conditions and rehabilitation, even after severe COVID-19 [24]. Finally, this study was a forerunner to an important bicentric study performed in Padova [25].

The bicentric study enrolled 41 patients, 21 with SARS-CoV-2 infection and 20 who were never infected by SARS-CoV-2 (as a control group) that underwent surgical resection for lung cancer [25]. Within the SARS-CoV-2 positive group, nine patients had COVID-19 pneumonia, and 12 did not. Lung tissue was collected at least 2 cm away from the tumor. All patients were SARS-CoV-2 negative for at least 30 days before surgery and required two negative RT-PCR results for SARS-CoV-2. The study found frequent and several lesions in the tumor-distant lung parenchyma of the SARS-CoV-2 positive group that had pneumonia. Major lesions included alveolar epithelial metaplasia, pleural fibrosis, and, albeit not statistically significant, more extensive peribronchial fibrosis than the SARS-CoV-2 negative group. The most frequent vascular lesions detected in the SARS-CoV-2 positive group are illustrated in the photomicrograph panel (Fig. 3). Additionally, genomic and subgenomic SARS-CoV-2 RNA transcripts were not detected in any sample, suggesting that the observed changes were not due to active viral replication but may instead have been long-term consequences of the infection. The authors concluded that investigating architectural changes in non-neoplastic lung tissue among recovered COVID-19 patients is extremely important as it could have a crucial overall impact on the future management of these frail patients [25].

In conclusion, the few available biopsy studies (Fig. 4) have provided valuable insights into histopathological alterations of post-COVID-19 lung pathology. We can summarize these findings in four key points:

1 Parenchymal damage exists even in asymptomatic or paucisymptomatic patients.
2 Heterogeneity is present in long COVID similarly to early lesions.
3 The viral genome can be detected (even in replicative status) in the tissue of patients with long COVID-19 despite a negative nasal swab for SARS-CoV-2.
4 Mild to moderate parenchymal and vessel remodelling appear to be the most frequent lesions.

Future studies need to develop a standardized approach to the pathological interpretation of lung biopsies especially with a strong focus on the use of ancillary tools like molecular pathology, digital pathology and artificial intelligence to increase sensitivity and standardize the pathological evaluation.

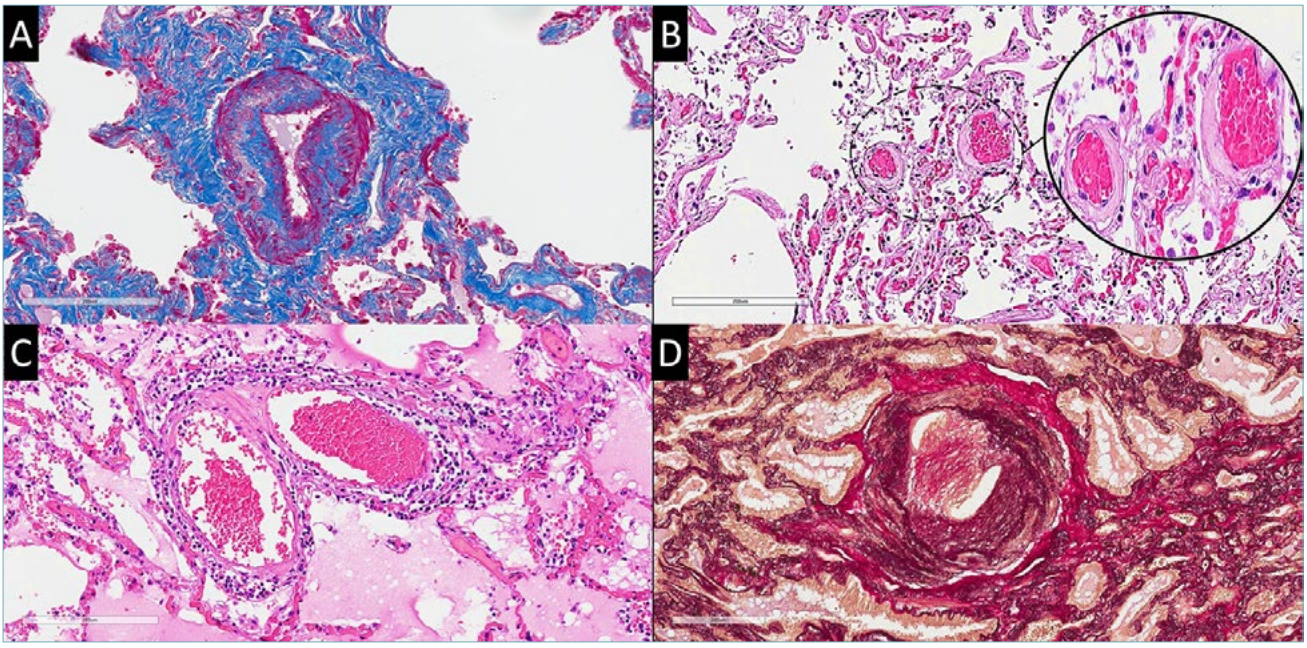


**Figure 3.** This panel illustrates four representative vascular lesions identified in the SARS-CoV-2 positive group (all images are at 20x magnification). A) Azan Mallory special stain highlights medial and intimal vascular. B) H&E stain emphasizes the hyalinization of the vascular wall – a zoomed-in inset is included. C) H&E stain showcases perivascular and vascular wall lymphoplasmacytic infiltrate, indicating vasculitis. D) Azan Mallory special stain depicts a blood vessel with lumen narrowing and recanalization.

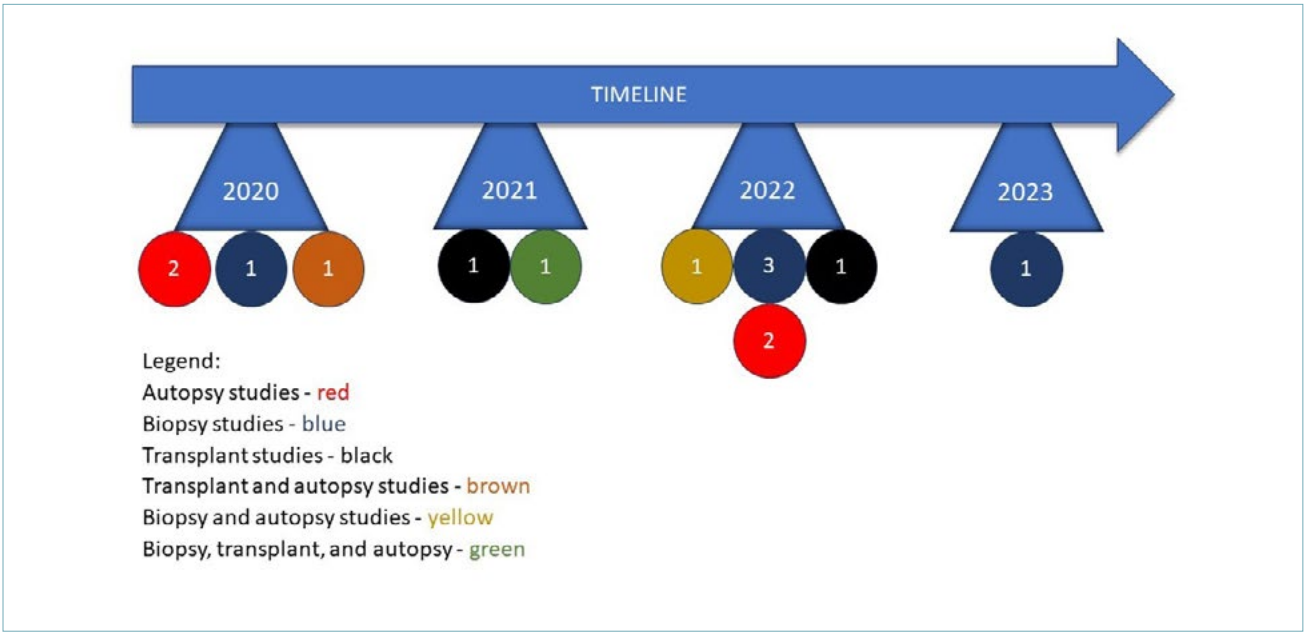


**Figure 4.** This graphic illustrates a timeline, tracking the annual count of studies conducted in relation to autopsies, transplants, and biopsies within the context of post-COVID-19 lung pathology.

## Acknowledgements

The authors would like to acknowledge all the people who suffered and are suffering because of the COVID-19 pandemic and the sacrifices that were made and are made by the scientific and medical community for the betterment of all.

## Conflicts of interest

Authors declare no potential conflicts of interest must also be explicitly stated.

## Funding

This work was partially supported by a fellowship from the University of Padova (Intesa San Paolo Vita bank) for young pathologists (EGO). Provided only support and did not play any role in the design, analysis and writing of the study (2020A08).

## Authors' contributions

All authors contributed equally to the conception, drafting, and editing of the manuscript.

## Ethical consideration

Ethical approval was not sought due to the non-experimental nature of the present work.

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