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Review

COVID-19 and H1N1-09: A Systematic Review of Two Pandemics with a Focus on the Lung at Autopsy

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Abstract

Background: The purpose of this manuscript is to provide a comparative overview of the two global pandemics: the first on June 11th 2009 due to influenza A H1N1 (H1N1-09); the second and current pandemic caused by coronavirus 2019 (COVID-19) on March 11th 2020, focusing on how autopsy can contribute to the definition of cellular pathology, to clinical pathology and, more generally, to public health. **Methods**: A systematic literature search selection was conducted on PubMed database on June 5, 2021, with this search strategy: (COVID-19) AND (H1N1 influenza) showing 101 results. The following inclusion criteria were selected: English language; published in a scholarly peer-reviewed journal; full-length articles were further elected. To further refine the research was to focus on the type of manuscript: review, systematic review, and meta-analysis. A critical appraisal of the collected studies was conducted, analyzing titles and abstracts, excluding the following topics: treatment, public health measures and perception of the general population or healthcare personnel about their quality of life. According to these procedures, 54 eligible studies were included in the present review. **Results**: Histopathological findings play a key role in understanding the pathophysiological mechanisms of diseases and, thus possible therapeutic approaches. The evidence on the thrombo-inflammatory mechanism underlying COVID-19 is growing to a much greater magnitude than the diffuse alveolar damage in common with H1N1-09; our study appears to be in line with these results. The prevailing scientific thinking to explain the morbidity and mortality of COVID-19 patients is that it elicits an exuberant immune reaction characterized by dysregulated cytokine production, known as a "cytokine storm". **Conclusions**: The histological and immunohistochemical pattern demonstrated similarities and differences between the infectious manifestations of the two pathogens, which justify empirical therapeutic approaches, in the first phase of the COVID-19 pandemic. Therefore, the previous pandemic should have taught us to promote a culture of clinical and forensic autopsies in order to provide timely evidence from integration among autopsy and clinical data for early adopting adequate therapies.

Keywords: COVID-19; H1N1-09; pathophysiology; cytokines; forensic; autopsy; histopathology

1. Introduction

Over the past decade, the World Health Organization (WHO) has been forced to declare two global pandemics: the first on June 11th 2009 due to influenza A H1N1 (H1N1-09); the second and current pandemic caused by coronavirus 2019 (COVID-19) on March 11th 2020 [1]. According to the WHO, "a pandemic is the worldwide spread of a new disease" [2], although this definition has been questioned and labelled "elusive" [3] as it would not exclude non-infectious diseases, and, above all, is strictly bound to the term "new" [4]. Stimulated by these considerations, a broader and more fitting definition is "an epidemic occurring worldwide, or over a very wide area, crossing international boundaries and usually affecting a large number of people".

The purpose of this manuscript is thus to provide a comparative overview of the two pandemics, focusing on how autopsy can contribute to the definition of cellular pathology, the clinical pathology of COVID-19 and, more generally, of public health.

This is because forensic practice has been too easily relegated to courtrooms or questions of justice, overlooking and sidelining its historical function to investigate what happens to organs and tissues following an insult and how these changes cause the onset and evo-lution of diseases or death, in addition to monitoring, through the study of cadavers, the adverse effects of treatments (including vaccines), the impact of diagnostic procedures on mortality, and health surveillance on the causes of death (which may also be of an in-fectious nature).

2. Materials and Methods

2.1 Eligibility Criteria

The present systematic review was carried out according to the Preferred Reporting Items for Systematic review and Meta-Analyses (PRISMA) standards [5].

Table 1. Inclusion and exclusion criteria of the selected studies, according to PRISMA guidelines.

Inclusion criteria	Exclusion criteria
English language	Treatment
Scholarly peer-reviewed journal publications	Public health measures
Full-length articles	Perception of the general population or healthcare personnel
Type of manuscript (review, systematic review, and meta- analysis)	

2.2 Search Criteria and Critical Appraisal

A systematic literature search selection (Fig. 1) was conducted on PubMed database on June 5, 2021, with this search strategy: (COVID-19) AND (H1N1 influenza) showing 101 results. To narrow this search, the following inclusion criteria were selected: English language; published in a scholarly peer-reviewed journal; full-length articles were further elected. Another useful criterion to further refine the research was to focus on the type of manuscript: review, systematic review, and meta-analysis (Table 1).

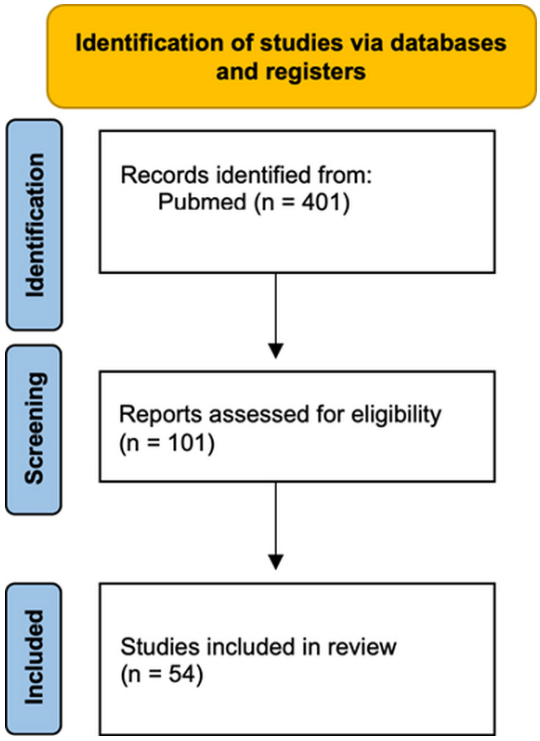

Fig. 1. Flow of the systematic review, according to PRISMA guidelines.

2.3 Search Results and Included Studies

A critical appraisal of the collected studies was conducted, analyzing titles and abstracts, as well as a hand search of reference lists were carried out by two researchers (GB and RLR) excluding the following topics: treatment, public health measures and perception of the general population or healthcare personnel about their quality of life. Data extraction was verified by another investigator (AM). According to these procedures, 54 eligible studies were included in the present review.

2.4 Risk of Bias

This systematic review concerns two pandemic events that occurred 10 years apart. Thus, it includes studies that were published in a time frame of 10 years. Therefore, study results should be interpreted taking into account that the accuracy of clinical and diagnostic procedure have changed over the years. As far as SARS-CoV-2 is concerned, it should be known that the dominant variant at the time of the study was: delta.

3. Results

3.1 Epidemiology

The H1N1-09 pandemic began in March 2009 in Mexico with subsequent diffusion in the US and then throughout the world until August 2010, with approximately 110 countries involved [1,6,7]. At the end of H1N1-09 pandemic, WHO confirmed 18,500 deaths, although, according to the study by Dawood *et al*. [8] these numbers were underestimated as the total number of deaths ranged between 151,700 and 575,400. The H1N1-09 virus continues to circulate in humans seasonally [9]. The current COVID-19 pandemic began in November-December 2019 in Wuhan, China, when several cases of pneumonia of unknown etiology were found. It subsequently spread to more than 203 countries and territories. As of 4 June 2021, a total of 171,708,011 cases worldwide have been confirmed since the start of the pandemic and 3,967,151 deaths and 33.689 deaths in Italy [10].

H1N1-09 virus infection (viral taxonomy: Riboviria › Orthornavirae › Negarnaviricota › Polyploviricotin› Insthoviricetes › Articulavirales › Orthomyxoviridae › Alphainfluenzavirus › Influenza A virus pH1N1) was a new strain of virus type A originating from the human, avian and pigvirus groups. Immediately after sequencing, the clinical condition generated by contact with this virus took the name of “swine flu”, because the viral strain was probably transmitted from pigs to humans, although pigs were not involved in the worldwide diffusion of the virus during the pandemic [11–14]. As regards the current pandemic, the etiological agent, whose genomic sequence was closely related to the SARS-CoV of 2003, has been identified as SARS-CoV-2 (viral taxonomy: Riboviria › Orthornavirae › Pisuviricota › Pisoniviricetes › Nidovirales › Cornidovirineae › Coronaviridae › Orthocoronavirinae ›

Betacoronavirus › Sarbecovirus › severe acute respiratory syndrome-related coronavirus-2) [12]. This virus likely originated in bats but may have been amplified in an intermediate host before transmission to humans represented by pangolins [13–15].

During pH1N1, the most affected age group included children and young adults with only 5% of cases relating to adults over the age of 51 [1,16,17], possibly due to partial immunity to the virus in the elderly population. Similarly, it was observed that the swine flu hospitalization rate decreases as the age of patients increases. The age group most affected by COVID-19 is adults over 40 (>70%), while only 10% of cases are under the age of 30 [1,15]. The COVID-19 hospitalization rate thus increases based on the age of the patients. This positive association shows that individuals over the age of 85 have the highest hospitalization and death rates [18].

As for pathogen transmission efficiency, influenza viruses and coronaviruses are both effective in causing respiratory diseases because they spread easily among humans through oral and nasal droplets. Furthermore, they can also be transmitted through indirect contact with infected surfaces (fomites) [19–21].

3.2 Pathophysiology

H1N1-09 is an enveloped-spherical virus, 80–120-nm in diameter, with a negative-polarity single-strand RNA, consisting of 8 segments coding for 12–14 proteins. The influenza virus infects the epithelial cells that line the upper respiratory tract (including the nasal tract) to the lower respiratory tract (up to the alveoli). Access to the cells of the upper respiratory tract is determined by the presence of sites rich in sialic acids, to which the hemagglutinin proteins present in human influenza viruses bind. These sites are particularly expressed in the soft palate [22]. The main mechanism of influenza pathophysiology is the result of lung inflammation and impairment caused by direct viral infection of the respiratory epithelium, combined with the effects of lung inflammation caused by host-triggered immune responses that limit the spread of the virus. Alveolar macrophages and endothelial cells appear to play a key role, as inducing exposure of cytokines and viral antigens to the endothelial layer can amplify inflammation, with endothelial cells constituting a major source of pro-inflammatory cytokines. Inflammation can thus progress and spread systemically and manifest itself as multi-organ failure, but these consequences are generally occurred after severe pulmonary impairment. The inability of the lung to perform its primary gas exchange function occurs through mechanisms of airway obstruction, loss of alveolar structure, loss of pulmonary epithelial integrity, and degradation of the extracellular matrix that maintains the structure of the lung [23,24].

The COVID-19 virus also has the characteristic of being enveloped and spherical, with a diameter of about 120 nm and a positive polarity single-strand RNA, consisting of 1 segment coding for 15 non-structural proteins, 4 structural proteins (spike, envelope, membrane glycoproteins, and nucleocapsid protein), 8 accessory proteins [25]. For entry into the host cell, this species of human coronavirus binds to the angiotensin 2 converting enzyme (ACE2); individual variation in the expression and/or polymorphisms of ACE2 can influence susceptibility to infection by the COVID-19 phenotype [26,27]. This interaction then triggers ACE2 endocytosis along with the COVID-19 virion and fusion of the viral membrane and host cell. Simultaneously, the viral spike protein is exposed to endosomal proteases which lead to its cleavage at two different sites: the first removes the S1 subunit, while the second occurs within the S2 subunit and causes exposure of the fusion peptide. The viral packet is thus released into the host cytoplasm, where it usurps the cellular mechanism producing new viral particles. In this perspective, lung tissue represents the ideal candidate for virus action due to its large surface area, which makes it highly susceptible to inhaled viruses and the conspicuous expression of ACE2 by Type II alveolar epithelial cells (pneumocytes) [28].

The development and progression of COVID-19 continue with major pathological mechanisms such as direct virus-induced cytotoxicity in ACE2-expressing cells, dysregulation of the RAAS (renin-angiotensin-aldosterone system) as a consequence of virus-mediated ACE2 down-regulation, dysregulation of immune responses, endothelial cell injury and thrombosis and fibrosis. This is because of the internalization of the virion-ACE2 promoting the accumulation of Ang II, with consequent production of receptors for TNF-α and IL-6 and activation of macrophages in a pro-inflammatory state, which evolves towards the well-known clinical condition of “cytokine storm” [29]. Furthermore, the virus nucleocapsid protein can interact with Smad3 to prevent apoptosis of infected host cells, promoting tissue fibrosis mediated by transforming growth factor (TGF)-β [30]. Type II alveolar epithelial cells renew themselves autonomously and express high levels of ACE2 and are thus constantly oriented towards viral entry and replication, which induces a vicious cycle of tissue damage and repair that can eventually result in areas being replaced, in turn responsible for the exchange of gases in non-functional fibrotic tissue.

3.3 Clinical Findings and Radiology

For pH1N1, the incubation period was 1.5–3 days, while the incubation period for COVID-19 is usually longer (2–14 days), averaging 5.2 days [31,32]. In both cases, fever and respiratory symptoms are the dominant clinical picture; dyspnea affects subjects with severe COVID-19 and pandemic H1N1-09 indifferently, followed by cough, fatigue, myalgia, arthralgia, and headache. Rhinorrhea, sore throat, thoracic pain, and sputum production were more common during the H1N1-09 pandemic, whereas dry cough, diarrhea, and vomit were more frequent among

COVID-19 patients [33].

Clinical specimens including a throat or nasopharyngeal swab, saliva, or lower respiratory tract aspirate sample are indicated for laboratory diagnosis of H1N1-09 and COVID-19 infections [34–36]. Additionally, whole blood or serum/plasma samples can be collected for seroconversion assessment. Rapid techniques for detecting the influenza virus include immunofluorescence and enzyme immunoassays. For both viruses, RT-PCR is the gold standard technique.

At imaging, the most significant differences between the two pathological pictures were more evident on CT examinations, where linear opacification, pleural thickening, vascular enlargement, and crazy-paving signs, are more severe with COVID-19 pneumonia, while bronchiectasis and pleural effusion, are more common in patients with H1N1-09 pneumonia. Other imaging findings, including peripheral or peribronchial and perivascular distribution, ground-glass opacity (GGO), consolidation, subpleural line, air bronchogram, and bronchial distortion, were not significantly different between the two patient groups [37–40] (Fig. 2, Ref. [40]).

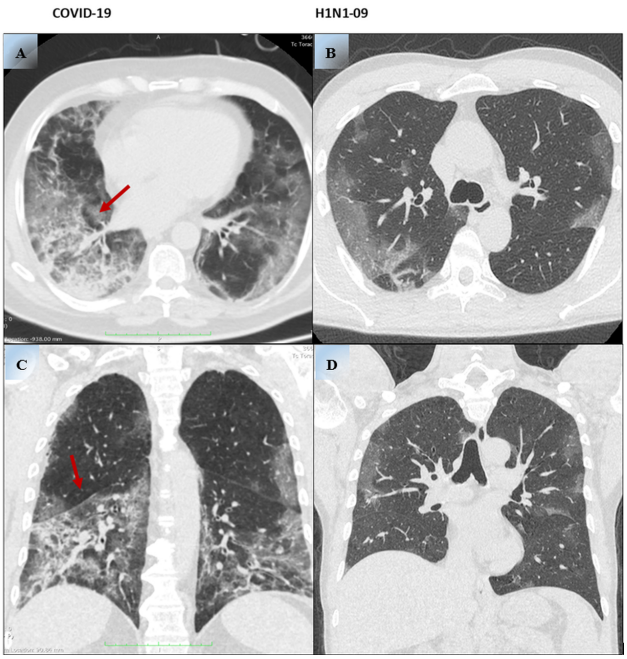

Fig. 2. Radiological pattern comparison. (A–C) with red arrow, the "crazy paving" sign. It consists of scattered or diffuse ground-glass attenuation with superimposed interlobular septal thickening and intralobular lines, the typical pattern among others of pathologies which pattern includes Pneumocystis carinii pneumonia, mucinous bronchioloalveolar carcinoma, pulmonary alveolar proteinosis, sarcoidosis, nonspecific interstitial pneumonia, organizing pneumonia, exogenous lipoid pneumonia [40]. (B–D) GGO pattern.

Regarding the radiological diagnosis of COVID-19, however, further clarification is required, as several studies have reported sensitivity of RT-PCR tests for SARS-CoV-2 between 37% and 83%, while chest sensitivity to CT for COVID-19 has been reported between 80% and 90% and specificity between 82.9% and 96% [41]. In this respect, in March 2020 a standardized disease diagnosis-probability system was proposed, the COVID-19 Reporting and Data System (CO-RADS), with a range that goes from CO-RADS 1 (COVID-19 is highly unlikely. CT is normal or findings indicate non-infectious diseases such as congestive heart failure, sarcoidosis, histoplasmosis, neoplasm, UIP or fibrotic NSIP) to CO-RADS 6 (Patient with PCR positive and bilateral GGO) [42].

3.4 Histological and Immunohistochemical Analysis

Histopathological analysis was performed on formalin-fixed paraffin-embedded (FFPE) lung samples collected by the same operator from two corpses with similar characteristics (Case 1: male between 50–60 years; mute history; positive swab for COVID-19 after entering the ER; died shortly after arriving at the hospital; Case 2: male between 50–60 years; mute history; positive swab for H1N1-09 after access to the emergency room; died 48 hours after entering the local hospital) during autoptic procedures performed at the Section of Legal Medicine, University of Foggia (Fig. 3).

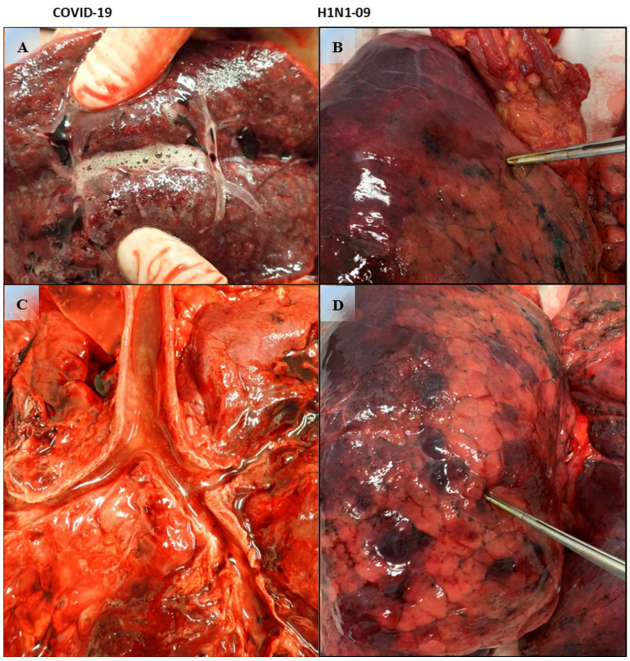

Fig. 3. Macroscopic findings. COVID-19: massive pulmonary edema at parenchymal level (A) and at airway opening (C). H1N1-09 (B–D): the forceps indicate widespread subpleural hemorrhages.

Standard hematoxylin and eosin (H&E) staining and modified Masson's trichrome stain [40] were performed (Fig. 4). For the immunohistochemical staining, after a first phase common to all the antibodies used and consisting in the preliminary pre-treatment for antigenic unmasking, subsequent application of the primary anti-staining antibody with Mayer's hematoxylin, the following anti-human antibodies were used: anti-nucleocapsid anti-COVID (anti Coronavirus -FIPV3-70 Santa Cruz Biotechnology, Inc., Dallas, TX, USA); anti-human fibrinogen (Dako A/S, Glostrup, Denmark); anti-human CD61 (Dako A/S, Glostrup, Denmark); anti-IL6 (Santa Cruz Biotechnology, Inc., Dallas, TX, USA); anti-HIF-1a.

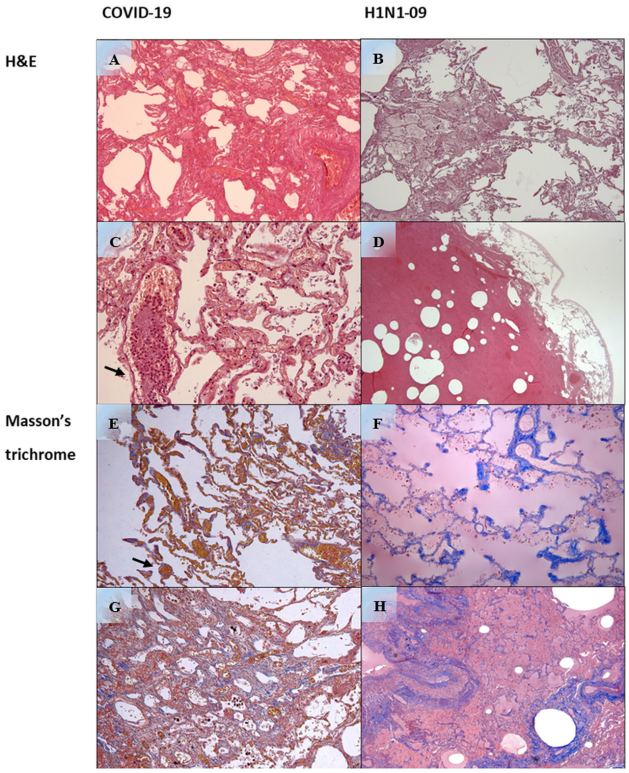

Fig. 4. Histopathological H&E staining and modified Masson's trichrome stain comparison. COVID-19 demonstrated the subacute organizing phase of DAD (A–G, 10×) with microthrombi (C–E, 20×) and H1N1-09 showed the acute or exudative phase with hyaline membranes (B, 20×) with edema (B, 10×–D, 20×–F, 20×), mild interstitial and alveolar inflammatory infiltrates, revealing a more marked involvement of the interstitium by the COVID-19 infection (H, 10×).

Furthermore, a qualitative method was used for the evaluation of each immunohistochemical staining [43–45].

The strength of the immunohistochemical staining weighed on different investigated areas was classified attributing values as follows: no detectable staining = 0, weak staining = 1, moderate staining = 2, strong staining = 3 (Fig. 5).

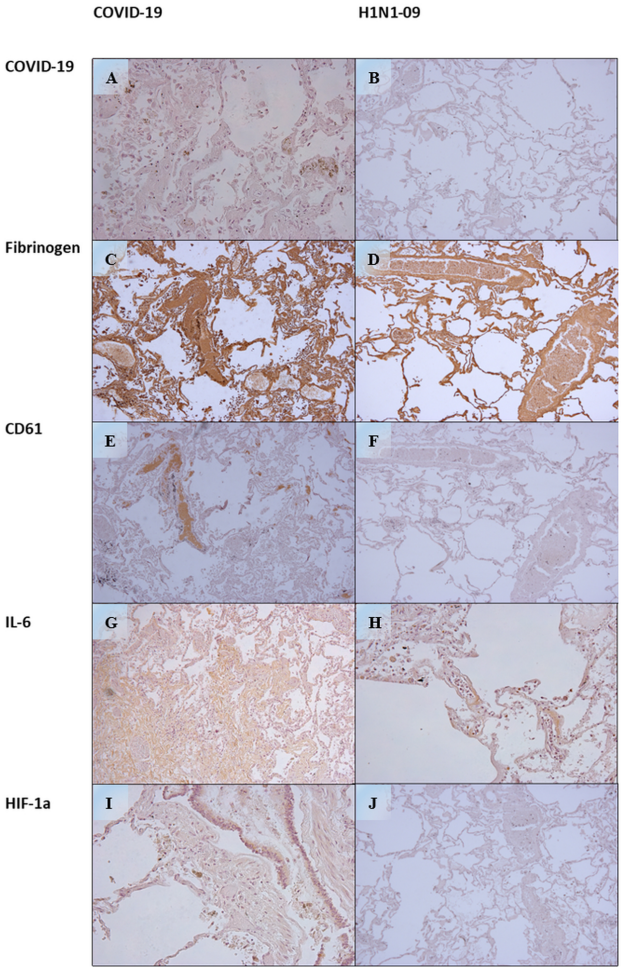

Fig. 5. Immunohistochemical comparison (20×). COVID-19 showed the following grading: (A) anti-COVID-19 = 2; (C) anti-human fibrinogen = 3; (E) anti-human CD61 = 3; (G) anti-IL6 = 2; (I) anti-HIF-1a = 2. H1N1-09 revealed the subsequent grading: (B) anti-COVID-19 = 0; (D) anti-human fibrinogen = 0; (F) anti-human CD61 = 0; (H) anti-IL6 = 1; (J) anti-HIF-1a = 0. These findings demonstrate that COVID-19 infection is associated with a greater extent of thromboinflammation than H1N1-09. This condition, which can be explained by a more marked tropism of the virus for endothelial cells, would in turn explain the deeper cellular hypoxia induced in cases of COVID-19 infection, demonstrated by the positivity to HIF-1a.

Diffuse Alveolar Damage (DAD) is the typical finding of the histological analysis of viral ARDS induced by COVID-19 and H1N1-09 and represents the severe form of the Acute Lung Injury (ALI) spectrum. DAD is caused by "lesions of the cells of the endothelial and alveolar lining that led to the exudation of liquids and cells", which culminates in the physical destruction of the alveolo-capillary membrane and, thus, in the inability to exchange gas [46].

Samples from autopsies conducted on individuals who died from H1N1-09 and COVID-19, demonstrated that the acute or exudative phase of DAD was the predominant

Table 2. Differences between COVID-19 and H1N1-09 infections.

	COVID-19	H1N1-09
Epidemiology	Zoonosis, from November-December 2019 still now; more than 203 countries involved [12–15]	Zoonosis, from March 2009 until August 2010; about 110 countries involved [11–14]
Age group most affected	adults over 40 y.o. [1,15,18]	children and young adults [1,16,17]
Transmission	oral and nasal droplets, fomites [19–21]	oral and nasal droplets, fomites [19–21]
Cell binding site	ACE-2 [26–28]	sites rich in sialic acids [22–24]
Incubation period	2–14 days (average 5.2 days) [31]	1.5–3 days [32]
Semeiotics	dry cough, diarrhea, and vomit [33]	Rhinorrhea, sore throat, thoracic pain, and mucus production [33]
Gold-standard diagnostic tool	RT-PCR [34–36]	RT-PCR [34–36]
CT imaging	GGO and crazy-paving sign [37–42]	bronchiectasis and pleural effusion [37–39]
Histology	DAD, macrophagic inflammatory infiltrates, associated with thickening of the alveolar walls; presence of organizing fibrosis; presence of microthrombi [37,47–55,57]	DAD, intra-alveolar inflammatory infiltrates, consisting of macrophages, polymorphonuclear cells and lymphocytes scattered between areas of edema and hemorrhage [47–49]

pulmonary histological picture (hyaline membranes with edema, mild interstitial inflammatory infiltrates and pneumocytes desquamated with reactive hyperplasia of pneumocytes) [47–49].

In detail, the histological changes most frequently found in the lungs during the H1N1-09 pandemic were intra-alveolar inflammatory infiltrates, consisting of macrophages, polymorphonuclear cells and lymphocytes scattered between areas of edema, hemorrhage and fibrin deposits. By contrast, inflammatory infiltrates found in the lungs of COVID-19 patients were dominated by macrophages, associated with thickening of the alveolar walls and partial loss of histological architecture [37]. In addition, in just over half the COVID-19 cases, the presence of organizing fibrosis has been described and reported in the histopathological examination, indicating an early transition to the subacute organizing phase. However, the important almost pathognomonic, feature of lung damage in COVID-19 patients is the presence of microthrombi [50, 51], resulting in speculation that COVID-19 has a predilection for endothelial cells. Vascular thrombosis and microthrombosis are frequent findings in DAD, resulting from local inflammation even in the absence of a state of systemic hypercoagulability, which occurs early on in ARDS of various causes, but would be more verifiable in cases of COVID-19 infection (Figs. 4,5). According to some authors, this phenomenon is nine times greater and it is associated with phenomena of neovascularization [52] and hemorrhages with different severity ranging [53]. Perivascular inflammation was also described as defining feature of the virus-induced systemic disease [54,55].

4. Conclusions

As demonstrated by the analysis conducted so far, "COVID-19 Is Not Comparable to H1N1 Influenza" [56] (Table 2, Ref. [1,11–24,26–28,31–42,47–55,57]) and histopathological findings play a key role in understanding the pathophysiological mechanisms of diseases and, thus possible therapeutic approaches.

While it is true on the one hand that this parallelism has the limitation of being retrospective, thus referring to two clinical conditions with a different genetic background and different sociomedical characteristics as regards the historical period and geographic areas involved, on the other hand comparison between the two pathologies and, above all, their differences, demonstrate that it is possible to ascertain various useful aspects, for physiopathological and therapeutic purposes. Today the evidence on the thromboinflammatory mechanism underlying COVID-19 is growing to a much greater magnitude than the diffuse alveolar damage in common with H1N1-09; our study appears to be in line with these results [58]. Furthermore, the immune reaction evoked by COVID-19 is still not well understood. The prevailing scientific thinking to explain the morbidity and mortality of COVID-19 patients is that it elicits an exuberant immune reaction characterized by dysregulated cytokine production, known as a "cytokine storm" [59,60].

The immune receptors that recognize the viral infection and initiate immune responses against the virus could be the toll-like receptors (TLRs) TLR3 and TLR4, which induce an immune reaction via the MyD88 and TRIF pathways. It is also possible that COVID-19 activates the inflammasome, as high levels of IL-1β have been observed in affected patients. Recently, however, the hypothesis has begun to emerge that this condition may be associated with a state of cellular immune depletion which includes monocytes, dendritic cells, CD4 + T cells, CD8 + T cells, B cells, and NK cells. These data suggest that severe COVID-19 is a state of immunosuppression similar to the well-known sepsis-induced immunosuppression [61,62]. In particular, a recent study by Remy *et al*. [63] showed that the immuno-

suppression observed in these subjects is even more profound than in critically ill patients with sepsis from other causes. Their research demonstrated that IFN-γ production generated by peripheral blood T cells of COVID-19 patients was reduced compared to T cells of healthy individuals and septic patients upon stimulation with anti-CD3/anti-CD28 antibodies, thus raising the suggestion that the primary immune mechanism underlying COVID-19 morbidity and mortality is immunosuppression rather than hyperinflammation. A recent study focused on endothelial damage between COVID-19, H1N1, and bacterial sepsis, demonstrating that myeloperoxidase levels were higher in COVID-19 patients, as well as ADAMTS-13 activity was greater than patients with H1N1 pneumonia or bacterial sepsis [64]. Furthermore, a study on post-mortem preparations would seem to confirm the role of oxidative stress in the pathogenesis of lethal forms of COVID-19, demonstrated by an abundant immunohistochemical expression of the lipid peroxidation product: 4-hydroxynonenal (4-HNE). The origin of 4-HNE would be vascular stress similar to sepsis and the organs most affected were the lungs with diffuse alveolar damage and the brain with edema and reactive astrocytosis [57]. A recent study attempted to define a unifying theory of the two souls of SARS-CoV-2 infection [65]. In response to viral septicemia, the host activates the complement system that produces the C5b-9 complement terminal complex to neutralize the pathogen. C5b-9 causes an increase in the permeability of the membrane of endothelial cells. to this is added the damage induced by the binding of the viral protein S to the endothelial ACE2 receptor. Both mechanisms produce endotheliopathy, which activates two molecular pathways: inflammatory and microthrombotic. In fact, the release of inflammatory cytokines and the endothelial exocytosis, of von Willebrand factor and FVIII, from the Weibel-Palade bodies occur. The recruitment of circulating platelets follows and, thus, microthrombogenesis begins. In COVID-19, microthrombosis initially affects the lungs by tropism causing ARDS.

The fact remains that the immune response to COVID-19 is completely different from the response to pandemic influenza A (H1N1). The pathogenicity and virulence of the H1N1-09 virus are due to the acquired properties that contribute to altering the regulation of inflammatory responses and evading antiviral immunity, downregulating the expression of cytokine signaling suppressors 1 (SOCS-1), and increasing the production of IL-6, IL-8, TNF-α [66]. H1N1-09 induces lower levels of Type I interferons in human macrophages and human lung epithelial cells. In this sense, it is possible that the virus blocks Type I interferon responses.

These pathopshysiolocial aspects could have influenced the initial therapeutic approach, considering that when H1N1-09 became a pandemic there were drugs that could be used for seasonal influences, such as oseltamivir and zanamivir [67]. On the other hand, when COVID-19 spread, there were no drugs or therapeutic protocols on the market to combat it [68,69]. Suffice it to say that in Italy the guidelines proposed by the body responsible for health care have not changed for a long period of time: Document of Ministry of Health, April 2021—Home management of patients with SARS-CoV-2 infection; Document of the Ministry of Health, November 2020—Home management of patients with SARS-CoV-2 infection. However, initially in an empirical form, later in a more structured model, low molecular weight heparins were added to the treatment protocols [70,71]. This could be related to the dysregulation of the coagulation cascade and subsequent clot formation common to all coronavirus infections associated with severe respiratory diseases (severe acute respiratory syndrome coronavirus 1, SARS-CoV-1, and the Middle East Respiratory Syndrome coronavirus, MERS-CoV), to be attributed, according to some authors, to the prothrombotic response, which attempts to prevent diffuse alveolar hemorrhage [71]. Such drugs could have been included earlier and effectively in therapeutic guidelines if there had been an understanding of the complex pathophysiological mechanisms of the infection, on which autopsy examinations could have shed light. The previous pandemic should have taught us to promote a culture of clinical and forensic autopsies [48] and both pandemics supports the need to provide a centralized, national and supra-national, infrastructure which promotes rapid review and integration among autopsy and clinical data to improve consciousness for efficient treatment strategies [72].

Abbreviations

ACE2, angiotensin 2 converting enzyme; ALI, Acute Lung Injury; CD, cluster of differentiation; CO-RADS, COVID-19 Reporting and Data System; COVID-19, COronaVIrus Disease-19; CT, computerized tomography; DAD, diffuse alveolar damage; GGO, ground-glass opacity; H&E, hematoxylin and eosin; IFN-γ, interferon-γ; IL-6, Interleukin 6; MyD88, Myeloid differentiation primary response gene (88); NK, natural killer; NSIP, non specific interstitial pneumonia; PRISMA, Preferred Reporting Items for Systematic review and Meta-Analyses; RAAS, renin-angiotensin-aldosterone system; RNA, RiboNucleic Acid; RT-PCR, Reverse transcriptase-polymerase chain reaction; Smad3, Mothers against decapentaplegic homolog 3; SOCS-1, cytokine signaling suppressors 1; TLR, toll-like receptor; TNF-α, tumour necrosis factor-α; TGF-β, tumour necrosis factor-β; TRIF, TIR-domain-containing adapter-inducing interferon-β; UIP, usual interstitial pneumonia; WHO, World Health Organization.

Author Contributions

GB, VF and PF designed the systematic review. MF and NDF performed the research. GB and RLR performed a critical appraisal. AM and GD performed data extraction. GB, MF and VF carried out post-mortem investiga-

tions. GB, MF and AM realized the original draft preparation. PF and RLR reviewed and edited the manuscript, under the supervision of VF. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All procedures performed in the study (forensic pathology material extraction and fixation) were in accordance with the ethical standards of the involved institutions and with the 1964 Helsinki Declaration and its later amendments. The processing of the data reported in this paper is covered by the general authorization to process personal data for scientific research purposes granted by the Italian Data Protection Authority (1 March 2012 as published in Italy's Official Journal no. 72 dated 26 March 2012) since the data do not entail any significant personalized impact on data subjects. Our study does not involve the application of experimental protocols; therefore it does not require approval by an institutional and/or licensing committee. In all cases, local prosecutors opened an investigation, ordering that an autopsy be performed to clarify the exact cause of death.

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Conflict of Interest

The authors declare no conflict of interest. RLR is serving as the guest editor of this journal. VF is serving as the editorial board member of this journal. We declare that RLR and VF had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to GP.

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