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# Autopsies' findings on COVID 19 patients: an alternative pathway to preserve patients' life.

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# ABSTRACT

Since 2019, SARS-CoV-2 (Severe Acute Respiratory Syndrome – CoronaVirus-2) infection spread globally reaching, according to WHO (World Health Organization) report of 18.10.2023, 771.407.825 confirmed infections and 6.972.152 deaths. A correctly implemented and widely accepted vaccination campaign was the only truly effective weapon to reduce mortality and hospitalizations related to COVID 19 (Coronavirus disease-19). However, even though more than 60% of the worldwide population is fully vaccinated (meaning that these subjects have completed the recommended vaccine cycle), subjects continue to die from COVID 19, particularly in the presence of comorbidities. In this scenario, autopsies play a crucial role in understanding the pathophysiological mechanisms of SARS-CoV-2 in vaccinated and no vaccinated subjects and adapting therapies and preventing strategies accordingly.

The main aim of this research project is focused on the identification and description of lesions in subjects who died with or from COVID 19, vaccinated or not. In particular, it is highlighted the importance of the microscopic findings (histological and immunohistochemical investigations) that play a pivotal role in the definition of the cause of death, allowing the identification of new singular histological lesions that could be related by the new variants of the virus.

A further goal of this study is to explore the relationship between the vaccination status of the patients and the COVID 19 variant that affected them, in order to define the protective efficacy of the vaccines and the relationship between the infection and the cause of the death, highlighting the importance of the vaccination campaign, as well as the importance of the virus variation monitoring the COVID 19 endemic phase.

# ACKNOWLEDGEMENTS

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# INTRODUCTION

In December 2019, a novel coronavirus caused a cluster of infectious diseases in Wuhan, China. In a matter of months, it spread around the world, prompting the World Health Organization (WHO) to declare a COVID-19 outbreak a Public Health Emergency of International Concern on January 30, 2020, and a pandemic on March 11, 2020.[1].

| **Timeline of the COVID 19 pandemic** | | |
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| **Outbreak** | **November 2019** | The outbreak was discovered in Wuhan, China. |
| | **December 2019** | Scientists reported the discovery of a novel coronavirus to the China CDC (CCDC) on 28 December[2].<br>On 31 December, the WHO office in China was notified about the cluster of unknown pneumonia cases and immediately launched an investigation.[3,4] |
| **First pandemic wave** | **January 2020** | On 11 January, WHO was notified by the Chinese National Health Commission that the outbreak was associated with exposures at the Huanan Seafood Wholesale Market, which also sold live animals, and that China had identified a new type of coronavirus, which it isolated on 7 January.[5]<br>In early and mid-January, the virus spread to other Chinese provinces, helped by the Chinese New Year migration. Wuhan was a transport hub and major rail interchange.[6]<br>On 30 January, 7,818 infections had been confirmed, leading WHO to declare the outbreak a Public Health Emergency of International Concern (PHEIC).[7] |

[1] Weiss, P., and Murdoch, D. R. (2020). Clinical course and mortality risk of severe COVID 19. The Lancet 395 (10229), 1014–1015. doi:10.1016/S0140-6736(20) 30633-4

[2] "China delayed releasing coronavirus info, frustrating WHO". Associated Press. 2 June 2020. Archived from the original on 25 October 2021. Retrieved 26 October 2021.

[3] "Mystery pneumonia virus probed in China". BBC News. 3 January 2020. Archived from the original on 5 January 2020. Retrieved 29 January 2020.

[4] "Novel Coronavirus". World Health Organization (WHO). Archived from the original on 22 January 2020. Retrieved 6 February 2020.

"COVID 19 timeline in the Western Pacific". World Health Organization (WHO). 18 May 2020. Archived from the original on 23 May 2020. Retrieved 6 July 2020.

[5] "Novel Coronavirus (2019-nCoV) SITUATION REPORT – 1". World Health Organization. 20 January 2020. Retrieved 7 June 2021.

[6] WHO–China Joint Mission (24 February 2020). "Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID 19)" (PDF). World Health Organization (WHO). Retrieved 8 March 2020.

[7] "Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV)". World Health Organization (WHO). 30 January 2020. Archived from the

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| | | By 31 January, Italy indicated its first confirmed infections had occurred, in two tourists from China, who had arrived in Italy on 23 January via Milan Malpensa Airport.[8]<br>On 16 February, a 38-year-old Italian went to Codogno Hospital (Lombardy), reporting respiratory problems, infecting other patients and health workers.[9] |
| | **February 2020** | In February the virus spread from Lombardy to the provinces of northern Italy. |
| | **March 2020** | On 8 March, Prime Minister Giuseppe Conte extended the quarantine lockdown to cover the whole region of Lombardy and 14 other northern provinces.[10]<br>On 10 March, Prime Minister Conte increased the quarantine lockdown to cover all of Italy, including travel restrictions and a ban on public gatherings.[11]<br>On 11 March, WHO announced its assessment that the situation could be characterized as a pandemic.[1]<br>On 19 March, Italy overtook China as the country with the most reported deaths.[12]<br>Three weeks into the lockdown, its effects began to show. Italy reported declines in the number of new cases and of new deaths per day. The country also saw a steady decrease in the occupancy of intensive care units.[13]<br>By 26 March, the United States had overtaken China and Italy as the country with the highest number of confirmed infections.[14]<br>On 31 March, the president of the Italian National Institute of Health announced that the pandemic had reached its peak in the country.[15] |

original on 31 January 2020. Retrieved 30 January 2020.

[8] Severgnini, Chiara (30 January 2020). "Coronavirus, primi due casi in Italia: sono due turisti cinesi". Corriere della Sera (in Italian). Retrieved 29 May 2023.

[9] "Codogno, i medici dell'ospedale in trincea: "Quelle accuse del premier fanno più male della malattia". la Repubblica (in Italian). 26 February 2020. Retrieved 26 February 2020

[10] "Coronavirus: Northern Italy quarantines 16 million people". BBC. 8 March 2020.

[11] "Coronavirus: Italy extends emergency measures nationwide". BBC. 10 March 2020.

[12] "Coronavirus: Number of COVID 19 deaths in Italy surpasses China as total reaches 3,405". Sky News. Retrieved 7 May 2020.

[13] "LATEST: Pressure on Italy's intensive care wards eases as new coronavirus cases slow again". The Local.it. 6 April 2020. Retrieved 7 April 2020.

[14] McNeil Jr DG (26 March 2020). "The U.S. Now Leads the World in Confirmed Coronavirus Cases". The New York Times. Retrieved 27 March 2020.

[15] "L'Italia ha raggiunto il picco", dice l'Istituto Superiore di Sanità. Agi (in Italian). 31 March 2020. Retrieved 7 April 2020.

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| | **May 2020** | In Italy, COVID 19 cases started to decline thanks to the two-months lockdown. Freedom of movements was re-established on 4 May and other not essential activities re-opened later in the month. |
| **Second pandemic wave** | **Sept 2020 – Oct 2020** | Since the end of September 2020, the virus regained strength and grew its prevalence in the regions of Campania and Lazio. This corresponded to a rise in new cases experienced also in other major European countries.<br>On 14 October, cases of COVID 19 positives exceeded the peak of the March infections.[16]<br>In October, WHO reported that one in ten people around the world may have been infected, or 780 million people, while only 35 million infections had been confirmed[17] |
| | **Nov 2020 – Jan 2021** | On 4 November 2020, Prime Minister Conte announced a new lockdown.<br>On 9 November, Pfizer released trial results for a candidate vaccine, showing a 90 percent effectiveness in preventing infection. That day, Novavax submitted an FDA Fast Track application for their vaccine.[18]<br>On 18 December, Public Health England reported that a variant had been discovered in the UK's southeast, predominantly in Kent. The variant, later named Alpha, showed changes to the spike protein that could make the virus more infectious.[19]<br>On 27 December, the vaccination campaign starts in Italy.<br>On 15 January, the Gamma variant was first identified in Japanese travelers returning from Brazil.[20] |
| **Third pandemic wave** | **Mar 2021 – May 2021** | On 12 March, several countries stopped using the Oxford-AstraZeneca COVID 19 vaccine- due to blood clotting problems, specifically cerebral venous sinus thrombosis (CVST).[21] |

[16] "COVID, nuovo record contagi: oltre 8 mila. Morti raddoppiano". ansa.it. 15 October 2020. Retrieved 15 October 2020.
[17] "One in 10 worldwide may have had COVID – WHO". BBC. 5 October 2020. Retrieved 14 October 2020.
[18] Boseley S, Olterman P. "COVID 19 vaccine candidate is 90% effective, says Pfizer". The Guardian. ISSN 0261-3077. Retrieved 9 November 2020.
[19] Le Page, Michael; McNamara, Alexander. "Alpha COVID 19 variant (B.1.1.7)". New Scientist. Retrieved 29 May 2023.
[20] "Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings". Virological. 12 January 2021. Retrieved 6 May 2021.
[21] "AstraZeneca defends COVID vaccine as handful of nations pause use over fear of blood clots". CBS News. Retrieved 14 March 2021.

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| | | On 6 May, the Delta variant was first identified in India.[22] On 26 November, the Omicron variant was detected in South Africa; a few days later the World Health Organization declared it a VoC (variant of concern).[23] |
| | **2022** | By 6 July, Omicron subvariants BA.4 and BA.5 had spread worldwide.[24] On 11 November, the WHO reported that deaths since the month of February had dropped 90 percent.[25] |
| **Endemic infection** | **2023** | On 5 May, the WHO downgraded COVID 19 from being a global health emergency, though it continued to refer to it as a pandemic.[26] The WHO does not make official declarations of when pandemics end[27], but by May, most countries had returned to daily life as it was before the pandemic.[28] |

## Virology

The COVId-19 disease of the year 2019 is due to the contamination with the intense acute respiratory syndrome virus number 2 (SARS-CoV-2), which primarily impacts the respiratory system. SARS-CoV2 is a part of the family of Coronaviridae that encompasses HCoV-39 229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43.[29]

Each SARS-CoV-2 virion is 60–140 nanometers (2.4×10−6–5.5×10−6 in) in diameter.[30,31]

[22] Callaway E (July 2021). "Delta coronavirus variant: scientists brace for impact". Nature. 595 (7865): 17–18. Bibcode:2021Natur.595...17C

[23] Fink, Jenni (22 December 2021). "Omicron variant that may resist vaccines found in all U.S. states". Newsweek. Retrieved 25 December 2021

[24] "BA.5, now dominant U.S. variant, may pose the biggest threat to immune protection yet". NBC News. 7 July 2022. Retrieved 13 August 2022.

[25] "WHO reports 90% drop in global COVID 19 deaths since February". MSN. Retrieved 11 November 2022.

[26] Nolen, Stephanie (5 May 2023). "W.H.O. Ends Global Health Emergency Designation for COVID". New York Times. Retrieved 5 May 2023

[27] Rigby, Jennifer (8 May 2023). "WHO declares end to COVID global health emergency". Reuters. Retrieved 9 May 2023.

[28] "From emergency response to long-term COVID 19 disease management: sustaining gains made during the COVID 19 pandemic". www.who.int. World Health Organization. Retrieved 9 May 2023.

[29] Shao N, Zhang C, Dong J, Sun L, Chen X, Xie Z, et al. Molecular evolution of human coronavirus-NL63, -229E, -HKU1 and – 482 OC43 in hospitalized children in China. Front Microbiol. 2022;13:1023847. doi: 10.3389/fmicb.2022.1023847. PubMed PMID: 483 36406425; PubMed Central PMCID: PMCPMC9666422.

[30] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. (February 2020). "A Novel Coronavirus from Patients with Pneumonia in China, 2019". The New England Journal of Medicine. 382 (8): 727–733. doi:10.1056/NEJMoa2001017

[31] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. (February 2020). "Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study". Lancet. 395 (10223): 507–513. doi:10.1016/S0140-6736(20)30211-7

Like other coronaviruses, SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins; the N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope.[32] Coronavirus S proteins are glycoproteins and also type I membrane proteins (membranes containing a single transmembrane domain oriented on the extracellular side).[33] They are divided into two functional parts (S1 and S2).[34] In SARS-CoV-2, the spike protein, which has been imaged at the atomic level using cryogenic electron microscopy,[35,36] is the protein responsible for allowing the virus to attach to and fuse with the membrane of a host cell[32]; specifically, its S1 subunit catalyzes attachment, the S2 subunit fusion.[37]

As of early 2022, about 7 million SARS-CoV-2 genomes had been sequenced and deposited into public databases and another 800,000 or so were added each month.[38] By September 2023, the GISAID EpiCoV database contained more than 16 million genome sequences.[39]

SARS-CoV-2 has a linear, positive-sense, single-stranded RNA genome about 30,000 bases long.[34] Its genome has a bias against cytosine (C) and guanine (G) nucleotides, like other coronaviruses.[40] The genome has the highest composition of U (32.2%), followed by A (29.9%), and a similar composition of G (19.6%) and C (18.3%).[41] The nucleotide bias arises from the

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[32] Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. (May 2020). "Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods". Acta Pharmaceutica Sinica B. 10 (5): 766–788. doi:10.1016/j.apsb.2020.02.008

[33] Jackson CB, Farzan M, Chen B, Choe H (January 2022). "Mechanisms of SARS-CoV-2 entry into cells". Nature Reviews Molecular Cell Biology. 23 (1): 3–20. doi:10.1038/s41580-021-00418-x

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[35] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. (March 2020). "Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation". Science. 367 (6483): 1260–1263. Bibcode:2020Sci...367.1260W. doi:10.1126/science.abb2507

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[39] "GISAID - gisaid.org". gisaid.org. Retrieved 16 September 2023.

[40] Kandeel M, Ibrahim A, Fayez M, Al-Nazawi M (June 2020). "From SARS and MERS CoVs to SARS-CoV-2: Moving toward more biased codon usage in viral structural and nonstructural genes". Journal of Medical Virology. 92 (6): 660–666. doi:10.1002/jmv.25754

[41] Hou W (September 2020). "Characterization of codon usage pattern in SARS-CoV-2". Virology Journal. 17 (1): 138. doi:10.1186/s12985-020-01395-x

mutation of guanines and cytosines to adenosines and uracils, respectively.[42] The mutation of CG dinucleotides is thought to arise to avoid the zinc finger antiviral protein related defense mechanism of cells,[43] and to lower the energy to unbind the genome during replication and translation (adenosine and uracil base pair via two hydrogen bonds, cytosine and guanine via three).[42] The depletion of CG dinucleotides in its genome has led the virus to have a noticeable codon usage bias. For instance, arginine's six different codons have a relative synonymous codon usage of AGA (2.67), CGU (1.46), AGG (.81), CGC (.58), CGA (.29), and CGG (.19).[41] A similar codon usage bias trend is seen in other SARS–related coronaviruses.[44]

Virus infections start when viral particles bind to host surface cellular receptors.[45] Protein modeling experiments on the spike protein of the virus soon suggested that SARS-CoV-2 has sufficient affinity to the receptor angiotensin converting enzyme 2 (ACE2) on human cells to use them as a mechanism of cell entry.[46] By 22 January 2020, a group in China working with the full virus genome and a group in the United States using reverse genetics methods independently and experimentally demonstrated that ACE2 could act as the receptor for SARS-CoV-2.[47,48,49] Studies have shown that SARS-CoV-2 has a higher affinity to human ACE2 than the original SARS

[42] Wang Y, Mao JM, Wang GD, Luo ZP, Yang L, Yao Q, Chen KP (July 2020). "Human SARS-CoV-2 has evolved to reduce CG dinucleotide in its open reading frames". Scientific Reports. 10 (1): 12331. Bibcode:2020NatSR..1012331W. doi:10.1038/s41598-020-69342-y

[43] Rice AM, Castillo Morales A, Ho AT, Mordstein C, Mühlhausen S, Watson S, et al. (January 2021). "Evidence for Strong Mutation Bias toward, and Selection against, U Content in SARS-CoV-2: Implications for Vaccine Design". Molecular Biology and Evolution. 38 (1): 67–83. doi:10.1093/molbev/msaa188

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[49] El Sahly HM. "Genomic Characterization of the 2019 Novel Coronavirus". The New England Journal of Medicine. Archived from the original on 17 February 2020. Retrieved 9 February 2020.

virus.[50,51]SARS-CoV-2 may also use basigin to assist in cell entry.[52]

Initial spike protein priming by transmembrane protease, serine 2 (TMPRSS2) is essential for entry of SARS-CoV-2.[53] The host protein neuropilin 1 (NRP1) may aid the virus in host cell entry using ACE2.[54] After a SARS-CoV-2 virion attaches to a target cell, the cell's TMPRSS2 cuts open the spike protein of the virus, exposing a fusion peptide in the S2 subunit, and the host receptor ACE2.[37] After fusion, an endosome forms around the virion, separating it from the rest of the host cell. The virion escapes when the pH of the endosome drops or when cathepsin, a host cysteine protease, cleaves it.[37] The virion then releases RNA into the cell and forces the cell to produce and disseminate copies of the virus, which infect more cells.[55]

SARS-CoV-2 produces at least three virulence factors that promote shedding of new virions from host cells and inhibit immune response.[32] Whether they include downregulation of ACE2, as seen in similar coronaviruses, remains under investigation (as of May 2020).[56]

## Variants

These viruses are fast in replication thanks to an RNA-dependent RNA polymerase and this make them do several errors which predisposes them to develop many mutations that cause neutralization of the immune system, antibody escape and reduction of the efficacy of vaccines. They are especially non-synonymous deletions of S-protein which gave origins to many dominant lineages in less than two years. SARS-CoV-2 variants are called, by WHO and by SIG (U.S. government SARS-CoV-2 Interagency Group)[57] VOC (Variants of concern), Omicron (B.1.1.529, BA.1, BA.1.1, BA.2, BA.3, BA.4 and BA.5 lineages], VOI (Variants of interest), VBM (Variants

[50] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. (March 2020). "Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation". Science. 367 (6483): 1260–1263. Bibcode:2020Sci...367.1260W. doi:10.1126/science.abb2507

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Being Monitored), Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Delta (B.1.617.2 and AY lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1),1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2) and VOHC (Variants Of High Concern). To date, no VOHC have been identified in the United States. Each variant classification includes the possible attributes of lower classes (for example, VOC includes the possible attributes of VOI). VOCs increase transmissibility or detrimental changes in epidemiology of COVID 19, increasing virulence, changing clinical presentation of the disease and reducing the effectiveness of public health and social measures or available diagnostics, vaccines, therapeutics. VOCs might require one or more appropriate public health actions, such as notification to WHO under the International Health Regulations, reporting to CDC (Center for Disease Control and Prevention), local or regional efforts to control spread, increased testing, or research to determine the effectiveness of vaccines and treatments against the variant. VOI are variants with predicted genetic mutations or which are known to affect viral characteristics. VBM is a variant where data indicates there is a potential or clear impact on approved or authorized medical countermeasures or that have been associated with more severe disease or increased transmission but are no longer detected, or are circulating at very low levels, in the United States. These variants do not pose a significant and imminent risk to public health in the United States. A VOI or a VOC may be downgraded to this list after a significant and sustained reduction in its national and regional proportions over time, or other evidence indicates that a variant does not pose significant risk to public health in the United States. These variants continue to be closely monitored to identify changes in their proportions. A VOHC has clear evidence that prevention measures or medical countermeasures (MCMs) have significantly reduced effectiveness relative to previously circulating variants.

Possible attributes of a VOHC:

In addition to the possible attributes of a VOC

- Impact on MCMs;
- Demonstrated failure of diagnostic test targets;
- Evidence to suggest a significant reduction in vaccine effectiveness, a disproportionately high number of infections in vaccinated persons, or very low vaccine-induced protection against severe disease;
- Significantly reduced susceptibility to multiple EUA or approved therapeutics;
- More severe clinical disease and increased hospitalizations

A VOHC would require notification to WHO under the International Health Regulations,

reporting to CDC, an announcement of strategies to prevent or contain transmission, and recommendations to update treatments and vaccines. Currently, no SARS-CoV-2 variants are designated as VOHC.

Omicron variant is an important VOC of the virus and it clearly is not a direct descendant of the Delta strains or earlier VOCs. Instead, it appears to have evolved in parallel[58] and is different from publicly shared SARS-CoV-2 genomes and hence it is hard to predict its closest relative. It likely diverged early from other strains[58]. The Omicron VOC could have circulated and evolved in a single immunocompromised human patient or a chronically infected COVID 19 patient over weeks or months with little surveillance or it might have evolved in a nonhuman species from which it recently spilt back into the human. The Omicron VOC might have high infectivity, but causes less severe symptoms than previous variants, and is likely able to escape immunity. The increase in variants of SARS-CoV-2 leads to an increase in the infectivity of the virus and a reduction in vaccine efficacy, both due to viral immuno-resistance and the physiological reduction of the antibody level against the virus. The increase in variants raises concerns and alarmism in international health systems because of their greater transmissibility[59] and their greater "immune evasion". In fact, the increase in infections and hospitalizations of vaccinated individuals probably derives from a combination of a decrease in vaccination efficacy over time, and a reduction in vaccination efficiency against new variants.[60]

## Symptoms

The symptoms of COVID 19 are variable depending on the type of variant contracted, ranging from mild symptoms to a potentially fatal illness.[61,62] Common symptoms include coughing, fever, loss of smell (anosmia) and taste (ageusia), with less common ones including headaches, nasal congestion and runny nose, muscle pain, sore throat, diarrhea, eye irritation,[63] and toes

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[59] Leung, K.; Wu, J.T. Managing Waning Vaccine Protection against SARS-CoV-2 Variants. Lancet 2022, 399, 2–3.

[60] Dejnirattisai, W.; Shaw, R.H.; Supasa, P.; Liu, C.; Stuart, A.S.; Pollard, A.J.; Liu, X.; Lambe, T.; Crook, D.; Stuart, D.I.; et al. Reduced Neutralisation of SARS-CoV-2 Omicron B.1.1.529 Variant by Post-Immunisation Serum. Lancet 2022, 399, 234–236.

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swelling or turning purple,[64] and in moderate to severe cases, breathing difficulties.[65] People with the COVID 19 infection may have different symptoms, and their symptoms may change over time. Three common clusters of symptoms have been identified: one respiratory symptom cluster with cough, sputum, shortness of breath, and fever; a musculoskeletal symptom cluster with muscle and joint pain, headache, and fatigue; and a cluster of digestive symptoms with abdominal pain, vomiting, and diarrhea.[65] In people without prior ear, nose, or throat disorders, loss of taste combined with loss of smell is associated with COVID 19 and is reported in as many as 88% of symptomatic cases.[66,67,68]

Of people who show symptoms, 90% develop only mild to moderate symptoms (up to mild pneumonia), while 9% develop severe symptoms (dyspnea, hypoxia, or more than 50% lung involvement on imaging) that require hospitalization, and 1% of patients develop critical symptoms (respiratory failure, septic shock, or multiorgan dysfunction) requiring ICU admission.[69] At least a third of the people who are infected with the virus do not develop noticeable symptoms at any point in time.[70,71] These asymptomatic carriers tend not to get tested and can still spread the disease.[71,72,73,74] Other infected people will develop symptoms later (called "pre-

Open Ophthalmology. 5 (1): e000632. doi:10.1136/bmjophth-2020-000632

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[69] "Interim Clinical Guidance for Management of Patients with Confirmed Coronavirus Disease (COVID 19)". U.S. Centers for Disease Control and Prevention (CDC). 6 April 2020. Archived from the original on 2 March 2020. Retrieved 19 April 2020.

[70] Oran DP, Topol EJ (May 2021). "The Proportion of SARS-CoV-2 Infections That Are Asymptomatic: A Systematic Review". Annals of Internal Medicine. 174 (5): 655–662. doi:10.7326/M20-6976

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[73] Lai CC, Liu YH, Wang CY, Wang YH, Hsueh SC, Yen MY, et al. (June 2020). "Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Facts and myths". Journal of Microbiology, Immunology, and Infection = Wei Mian Yu Gan Ran Za Zhi. 53 (3): 404–412. doi:10.1016/j.jmii.2020.02.012

[74] Furukawa NW, Brooks JT, Sobel J (July 2020). "Evidence Supporting Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 While Presymptomatic or Asymptomatic". Emerging Infectious Diseases. 26 (7).

symptomatic") or have very mild symptoms and can also spread the virus.[74]

As is common with infections, there is a delay between the moment a person first becomes infected and the appearance of the first symptoms. The median delay for COVID 19 is four to five days[75] possibly being infectious on 1-4 of those days.[76] Most symptomatic people experience symptoms within two to seven days after exposure, and almost all will experience at least one symptom within 12 days.[75,77]

Most people recover from the acute phase of the disease. However, some people continue to experience a range of effects, such as fatigue, for months, even after recovery.[78] This is the result of a condition called long COVID, which can be described as a range of persistent symptoms that continue for weeks or months at a time.[79] Long-term damage to organs has also been observed after the onset of COVID 19. Multi-year studies are underway to further investigate the potential long-term effects of the disease.[80]

## Vaccine

The Omicron variant became dominant in the U.S. in December 2021. Symptoms with the Omicron variant is less severe than they are with other variants.[81]

According to the importance of vaccination great effort and resources have been invested in developing vaccines since the beginning of the infection spreading.

Originally authorized and still usable vaccines in EU (European Union) are, according to EMA (European Medicines Agency):

- Comirnaty (developed by BioNTech and Pfizer) (Conditional marketing authorization issued: 21/12/2020)

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doi:10.3201/eid2607.201595

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[76] Byrne AW, McEvoy D, Collins AB, Hunt K, Casey M, Barber A, et al. (August 2020). "Inferred duration of infectious period of SARS-CoV-2: rapid scoping review and analysis of available evidence for asymptomatic and symptomatic COVID 19 cases". BMJ Open. 10 (8): e039856. doi:10.1136/bmjopen-2020-039856

[77] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC (August 2020). "Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID 19): A Review". JAMA. 324 (8): 782–793. doi:10.1001/jama.2020.12839

[78] "Half of young adults with COVID 19 had persistent symptoms after 6 months". medicalxpress.com. Retrieved 10 July 2021.

[79] CDC (1 September 2022). "Post-COVID Conditions". Centers for Disease Control and Prevention. Retrieved 21 September 2022.

[80] CDC (11 February 2020). "COVID 19 and Your Health". Centers for Disease Control and Prevention. Retrieved 23 January 2021.

[81] CDC (29 March 2022). "Omicron Variant: What You Need to Know". Centers for Disease Control and Prevention. Retrieved 15 June 2022.

- COVID 19 Vaccine (inactivated, adjuvanted) Valneva (Marketing authorization issued: 24/06/2022)
- Jcovden (previously COVID 19 Vaccine Janssen) (Conditional marketing authorization issued: 11/03/2021)
- Nuvaxovid (Conditional marketing authorization issued: 20/12/2021)
- Spikevax (previously COVID 19 Vaccine Moderna) (Conditional marketing authorization issued: 06/01/2021)
- Vaxzevria (previously COVID 19 Vaccine AstraZeneca) (Conditional marketing authorization issued: 29/01/2021)
- VidPrevtyn Beta (Marketing authorization issued: 10/11/2022)

It's possible to see, by the above-mentioned list, that some of these vaccines have obtained marketing authorization recently and this is indicative that the research follows variations in the virus and its ability to immune escape. COVID 19 vaccine efficiency estimates range from 55 to 70% after the first dose, with little variation by vaccine or age group[82]. At first, the double dose of the COVID 19 vaccine it has been reported had an efficacy varying between approximately 65% and 95%, producing a reduction in hospitalization of 75–85% and a reduction in mortality of 95–99%. A 35–50% reduction in transmission and risk of SARS-CoV-2 positivity was also found[83,84]. According to different authors, 14/20 days after the first dose, the effectiveness of the COVID 19 vaccine was about 46%, with a reduction in symptomatic disease of about 57%, hospitalizations were reduced by approximately 74%, severe disease by around 62%, and mortality by 72%.[85] On the other hand, 7 days after the second dose, the efficacy of the COVID 19 vaccine for documented infections was around 92%, 94% for symptomatic disease, 87% for the reduction in hospitalization, and 92% for severe disease[86]. For example, in a study performed in Chile, out of a cohort of 10.2 million people, the effectiveness of the vaccine was 66%, with a

[82] Lopez Bernal, J.; Andrews, N.; Gower, C.; Robertson, C.; Stowe, J.; Tessier, E.; Simmons, R.; Cottrell, S.; Roberts, R.; O'Doherty, M.; et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca Vaccines on COVID 19 Related Symptoms, Hospital Admissions, and Mortality in Older Adults in England: Test Negative Case-Control Study. BMJ 2021, 373, n1088

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[85] Dagan, N.; Barda, N.; Kepten, E.; Miron, O.; Perchik, S.; Katz, M.A.; Hernán, M.A.; Lipsitch, M.; Reis, B.; Balicer, R.D. BNT162b2 MRNA COVID 19 Vaccine in a Nationwide Mass Vaccination Setting. N. Engl. J. Med. 2021, 384, 1412–1423.

[86] Harris, R.J.; Hall, J.A.; Zaidi, A.; Andrews, N.J.; Dunbar, J.K.; Dabrera, G. Effect of Vaccination on Household Transmission of SARS-CoV-2 in England. N. Engl. J. Med. 2021, 385, 759–760

90% reduction in hospitalization, and an 86.3% reduction in mortality.[87]

[87] Jara, A.; Undurraga, E.A.; González, C.; Paredes, F.; Fontecilla, T.; Jara, G.; Pizarro, A.; Acevedo, J.; Leo, K.; Leon, F.; et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N. Engl. J. Med. 2021, 385, 875–884

# AIM OF THE STUDY

Since the start of the COVID-19 pandemic in 2019, the scientific community has been working on generating guidelines and recommendations to assist healthcare professionals in safely managing and investigating the disease. Various governments implemented strict regulations regarding the handling of deceased individuals who passed away from or with COVID-19, leading to significant ethical concerns. For instance, Italy, the first European country impacted by the COVID-19 outbreak, promptly prohibited burial procedures based on initial studies indicating the virus's environmental stability. Initially, cremation was encouraged for COVID-19 fatalities. Similar recommendations were issued by the Autopsy Work Group of the Spanish Society of Anatomical Pathology and the Royal College of Pathologists, discouraging autopsies in positive COVID-19 cases[88]. In contrast, the restriction on conducting autopsies during the COVID-19 pandemic hindered the collection of data on the novel virus. that discouraged to perform autopsies in positive COVID 19 cases. On the contrary, the restriction of autopsies during the COVID 19 pandemic has slowed down the acquisition of data about the new virus.

The primary objective of this research initiative is to illustrate that when appropriate precautions are implemented, autopsy is a secure operation with minimal infection risk for everyone involved, such as pathologists, technical personnel, and others. Additionally, this study aims to describe the histological abnormalities encountered in subjects who died from or with COVID-19. It is emphasized that histological and immunohistochemical investigations are crucial in determining the cause of death, as they enable the identification of histological alterations that may be related to the viral infection, thereby contributing to a better understanding of the disease's pathology.

[88] Osborn, M.; Lucas, S.B.; Stewart, R.; Swift, B.; Youd, E. Autopsy Practice Relating to Possible Cases of COVID 19 (2019-nCov, Novel Coronavirus from China 2019/2020).)

# MATERIALS AND METHODS

## Study design

This was a prospective study conducted in at the A.O.U. Policlinico "G. Rodolico – San Marco" in Catania, between April 2020 and April 2021 in Italy. The study was approved by Hospital local ethics committee (code: 28_09_2020_CT), and all procedures performed in the study were approved by the Scientific Committee of the Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", University of Catania, (record n. 21/2020) and were performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was conducted according to the Italian Law n◦ 81/2008 concerning the safety of workers and workplace of public Hospitals. The Director of San Marco Hospital authorized the use of anonymous data according to Italian law. No informed consent is required to use information from deceased persons where the same information is strictly indispensable and relevant for scientific and research purposes.

## Cohort Study

Patient demographic, epidemiological and clinical information was extracted from the case records of COVID 19 patients who died and where hospital or judicial autopsies were required.

Of a total of 35 autopsies were performed on patients who died with or from COVID 19, in our institution from April 2020 to April 2021, we selected 16 clinical and forensic autopsies of COVID 19 patients that satisfied the inclusion criteria: positivity to RT-PCR test for SARS-CoV-2 infection at the admission in Hospital; positivity to RT-PCR test for SARS-CoV-2 infection at the time of death; positivity to RT-PCR test for SARS-CoV-2 infection for lung tissue sampled during autopsy.

Before autopsy, both molecular and antigen tests were performed on all personnel involved in the post-mortem procedures obtaining negative results. Moreover, during the study period, they were constantly monitored (as programmed by our University, every 5 days) resulting negative at all steps.

## Autopsy procedures

Autopsies were performed using the specific guidance for post-mortem and collection detailed in the study protocol to reduce the risk of transmission of infectious pathogens during and after post-mortem examination. They were performed with a different post-mortem interval (PMI): 8 samples constituted the short PMI group (12 h $\leq$ PMI $\leq$ 72 h), meaning that the autopsies were

performed within 72 h, and 8 samples for the long PMI group (24 days ≤ PMI ≤ 78 days), meaning that the autopsies were performed on exhumed corpses after the indicated PMI; during this period, they were buried in galvanized coffins.

To assess the efficacy of the autopsy safety procedure in deceased COVID 19 positive subjects, and the effectiveness of the disinfection procedure, a standardized pre- and post-disinfection swab collection procedure was performed. The tool used for swab collection was in accordance with the CDC guidelines. The standardized procedure was divided into three stages:

T0—before the autopsy;

T1—at the end of autopsy (without removing the corpse);

T2—after the autopsy (after cleaning and disinfection of the AR).

With cadaver outside of the AR, environmental swabs were collected from specific points in the room. Autopsies were performed in biosafety level 3 (BSL3) or equivalent autopsy rooms (compliant with Centers for Disease Control and Prevention [CDC] guidelines and recommendations of the Italian Ministry of Health)[89,90,91,107] with airflow control and airborne infection control procedures, including use of appropriate personal protective equipment. A consensual specific protocol was approved before the start of this prospective study. The protocol included all steps from the introduction of the body to the autopsy room, to the end of the postmortem analysis. One method was allowed: organ extraction and sampling. The autopsy was conducted according to the *Letulle* technique[92], thus reducing environmental contamination. This technique consisted of carrying out an *en bloc* resection of all the cervical, thoracic, and abdominal organs to prevent the aerosolization of potentially contaminated biological fluids. The skull was opened by coronal cutting using a handsaw with a chain-mail glove to prevent bone and fluids aerosolization. Before death, all nasopharyngeal swabs collected from the subjects enrolled in the present study tested positive at the COVID 19 rRT-PCR assay[93]. During the autopsy, a lung swab

[89] Centers for Disease Control and Prevention Collection and Submission of Postmortem Specimens from Deceased Persons with Known or Suspected COVID 19. Available online: https://eaaf.org/wp-content/uploads/covid19PDFs/EEUU/CDC-guidance-postmortem-specimens.pdf (accessed on 18 August 2020).

[90] WHO Interm Guidance Infection Prevention and Control for the safe management of a dead body in the context of COVID 19. J. Hosp. Infect. 2020, 104, 246–251.

[91] Corpuz, J.C.G. A dignified death: Management of dead bodies during COVID 19. J. Public Health 2021.

[92] Pomara, C.; Fineschi, V. Forensic and Clinical Forensic Autopsy. An Atlas and Handbook, 2nd ed.; Pomara, C., Fineschi, V., Eds.; CRC

Press: Boca Raton, FL, USA, 2020; ISBN 9780367330712.

[93] Pomara, C.; Salerno, M.; Sessa, F.; Esposito, M.; Barchitta, M.; Ledda, C.; Grassi, P.; Liberto, A.; Mattaliano, A.R.; Rapisarda,V.; et al. Safe Management Strategies in Clinical Forensic Autopsies of Confirmed COVID 19 Cases. Diagnostics 2021, 11, 457.

sample was collected for each subject, confirming a positive result. Prior to fixation, three tissue fragments, from the right lung and two from the left lung, were collected and immediately transferred to sterile vials containing RNA Later (Cat. 76104, RNA Protect Tissue Reagent, Qiagen) and stored at −80 ◦C pending extraction and 2 swabs were taken from the lower respiratory tract (primary bronchi), the first to the right bronchus and the second to the left bronchus. All tissues were sent to a biosafety level 3 (BSL-3) laboratory for viral culture.

Before the cleaning and disinfection operation, 11 environmental samples were collected for each of 16 autopsies (176 total swab).

When the cadaver was outside the autopsy room, according to guidelines, the disinfection procedure was performed with a minimum concentration of 0,01% (1000 ppm) sodium hypochlorite (bleach). Moreover, the complete disinfection of the personnel involved in the autopsy procedures was performed before leaving the room at the end of the autopsy through a nebulization procedure of all PPE products (such as overalls, gloves, face shield, etc). After disinfection, 11 environmental swabs were also collected.

## Virus Isolation

For SARS-CoV-2 isolation, the Vero E6 cell line (African green monkey kidney cells) was used.[94] Cells were cultured in Eagle's minimal essential medium (EMEM) (Life Technologies, Carisbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Life Technologies, Carisbad, CA, USA), and 100 U/mL penicillin and streptomycin (Life Technologies, Carisbad, CA, USA). For the virus isolation from lung tissues, cells were plated into 25 cm2 cell culture flasks (Corning, New York, NY, USA) at a confluence of 70–80% in 6 mL EMEM with 10% FBS and incubated overnight at 37 ◦C. The following day, lung samples were mechanically homogenized by TissueRuptor II (Qiagen, Hilden, Germany) in 3 mL of PBS. Each sample was centrifugated at 5000× g for 5 min and the supernatant was filtrated at 0.8 µm and 0.22 µm (Sartorius Stedim Biotech, Aubagne, France). The filtrate was incubated with an equal volume of an antibiotic solution (2000 U/mL of penicillin/streptomycin and 300 U/mL of neomycin) for 1 h at room temperature. The suspension was then inoculated on the monolayer of the VeroE6 cells, and the flask was incubated at 37 ◦C for 1 h. After incubation, 5 mL of EMEM with 6% fetal bovine serum (FBS) was added and incubated again at 37 ◦C for 72 h. After 72 h, 200 µL of EMEM were collected from each flask for biomolecular testing and the EMEM 6% FBS was

[94] Rondinone, V.; Pace, L.; Fasanella, A.; Manzulli, V.; Parisi, A.; Capobianchi, M.R.; Ostuni, A.; Chironna, M.; Caprioli, E.; Labonia M.; et al. VOC 202012/01 Variant Is Effectively Neutralized by Antibodies Produced by Patients Infected before Its Diffusion in Italy. Viruses 2021, 13, 276

replaced after a further 72 h. At the end of the test a further 200 µL of flask medium was collected for the evaluation of viral load, while the flasks were observed under an inverted microscope Axiovert 25 (Zeiss, Oberkocken, Germany) to evaluate the presence of cytopathic effects.[95] The result was defined on the basis of the cytopathic effect (subjective reading) combined with the results of the RT-PCR test (objective reading) in supernatants.[96] All procedures for viral culture followed laboratory biosafety guidelines and were performed in a biosafety level 3 (BSL-3) laboratory. Viral RiboNucleic Acid (RNA) was extracted from the medium of flasks at T0, after 72 h and after 144 h, using the QIAamp Viral RNA Mini Kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Amplification and detection of target genes (N, E, and RdRP) were performed using the commercially available kit GeneFinder COVID 19 Plus RealAmp (Osang Healthcare Co. Ltd., Anyang, Korea) with the CFX96TM instrument (Bio-Rad, Hercules, CA, USA). The cycle threshold (Ct) of each RT-PCR reaction was calculated following the manufacturer's instructions. The test was considered positive when at least one of the three investigated genes showed a Ct below 40.[95]

## Tissue samples and histological staining

Tissues from the lung, trachea, heart, liver, kidney, spleen, central nervous system, testicles, and skin were collected. Samples were processed in two different ways according to their subsequent use: fresh samples or samples in 10% formaldehyde solution for optical microscopy and histopathological assessment. Tissue samples for optical microscopy were processed using hematoxylin and eosin staining (H&E). Special stains or immunohistochemical stains were performed locally and guided by histological findings in each case.

## Swabs analysis

All the swabs collected during the first 9 autopsies (cadaver ID 1-9, Table 1) were analyzed using the Aptima SARS-CoV-2 Assay (Hologic, Inc., San Diego, CA, USA), according to the manufacturer's instruction, with automatic data system analysis software (Panther Fusion, Hologic, Marlborough, MA, USA) for identifying positive samples. The other collected swabs (cadaver ID 10-16) were analyzed by multiplex rRT-PCR assay using GeneXpert Xpress SARS-CoV-2 on the CFX96 real-time (Cepheid, Sunnyvale, CA, USA). Both assays are designed to

[95] Manzulli, bghbV.; Scioscia, G.; Giganti, G.; Capobianchi, M.R.; Lacedonia, D.; Pace, L.; Cipolletta, D.; Tondo, P.; De Nittis, R.; Rondinone, V.; et al. Real Time PCR and Culture-Based Virus Isolation Test in Clinically Recovered Patients: Is the Subject Still Infectious for SARS-CoV2? J. Clin. Med. 2021, 10, 309

[96] Lenoci, G.; Galante, D.; Ceci, E.; Manzulli, V.; Moramarco, A.M.; Chiaromonte, A.; Labarile, G.; Lattarulo, S.; Resta, A.; Pace, L.; et al. SARS-CoV-2 isolation from a 10-day-old newborn in Italy: A case report. IDCases 2020, 22, e00960.

investigate genes specific for SARS-CoV-2 according to the US and Chinese Centers for Disease Control; moreover, a recent study demonstrate an overall agreement of 99% between the Cepheid Xpert Xpress SARS-CoV-2 assay and the GeneXpert Xpress SARS-CoV-2 assay confirming their position as robust and comparable diagnostic options for the identification of SARS-CoV-2. All the procedures to prevent specimen contamination and PCR carryover were rigorously respected at all phases.

## Results

### Patient baseline characteristics

Between April 2020 and April 2021, a total of 36 necropsies were performed. Out of the 16 patients included, 50% were men (n = 8); median age was 76.5 (range 50–93) years. Every patient had at least one comorbidity prior to COVID 19 diagnosis, the most frequent being the presence of vascular risk factors. Symptoms and main blood test parameters at baseline were assessed in 16 (100%) out of the 16 patients. Among symptoms, fever and dyspnea were most frequently reported. In blood tests, abnormalities in partial pressure of oxygen (PaO2), lactate dehydrogenase (LDH), C-reactive protein (CRP), D-dimer, ferritin and interleukin-6 levels were observed in most of the samples analyzed. Chest X-ray was performed in 100% (n = 16) of the patients.

### Autopsy findings

Complete necropsies were carried out in every patient. 10 organs per necropsy were analyzed (the lung, heart, liver, kidney, spleen, central nervous system, small bowl, testicles, lymph nodes and skin). As observed, COVID 19 is a systemic disease causing multiorgan damage. The most frequently affected organ was the lung (16 affected cases out of the 16 lungs analyzed, 100%) The main cause of death was interstitial pneumonia with fibrosis that involved five subjects cadaver (ID 1,6,8,10,14); moreover, four patients died from pulmonary edema (cadaver ID 2,4,5,12), three patients died from Multiple Organ Dysfunction Syndrome (MODS) (cadaver ID 13, 15,16), two from septic shock (cadaver ID 7,11), patient #9 died from cardiac failure, and patient #3 died from Acute respiratory distress syndrome (ARDS).

### Histological and Immunohistochemical findings

Histopathological findings were observed in 50% (8/16) of the hearts analyzed. The gross findings from the heart showed left ventricular hypertrophy. 2 clear cases of acute myocarditis were identified. In the central nervous system (CNS) only 8 cases were available. No vasculitis was reported and increased microglia was observed. In 8 cases (50%) findings were compatible with pre-infection arteriosclerosis; 4 cases in the heart, 2 in the kidneys and 2in both. Regarding the kidney, glomerular sclerosis was identified as the most frequent pre-infection

histopathological finding. In the liver, the presence of steatosis (25%, n = 4) was the most frequent findings reported. Other findings included inflammation, edema, and metaplasia in the trachea and fiber necrosis in the muscle.

**RNA detection and virus vitality**

The molecular test for SARS-CoV-2-RNA using quantitative rRT-PCR was performed on all samples of low respiratory airways collected at time T1 (at the end of autopsy). All 32 swabs (16 on the right bronchus and 16 on the left bronchus) were positive. The positive results were not influenced by the period of time (ranging from 5 to 54 days) elapsed between the ante-mortem COVID 19 diagnostic test and the post-mortem swabs (COVID 19 rRT-PCR assay performed on post-mortem swab). The crucial aspect is the persistence of the RNA virus in all decomposed bodies up to a PMI of 78 days. At time T0 (before the autopsy) and T2 (after the disinfection procedure) all environmental swabs collected from the autopsy room (total environmental swabs 176) were negative for the RNA of SARS-CoV-2. At time T1 (at the end of autopsy), the environmental swabs of the autopsy table were positive for the RNA of SARS-CoV-2. The Face shild swabs of the two forensic pathologists who performed the autopsy gave a positive rate of 15.6% (n = 5/32).

Furthermore, even if all patients tested positive at RT-PCR for the SARS-CoV-2 infection before death and at the time of the autopsy, we found differences in RT-PCR positivity between lung swabs and homogenized lung tissues where15 samples were negative, demonstrating that the positivity to the swab sample does not demonstrate virus vitality.

Contrariwise, it was demonstrated that 24 h after death, in the main site of infection (lung tissue), the virus was inactive and not able to infect. Finally, our study, in agreement with other international studies, also confirms that, with appropriate safeguards, autopsies of people who have died from COVID 19 can be performed safely and provide relevant information to medical research. [139,97]

[97] Boor P, Eichhorn P, Hartmann A, Lax SF, Märkl B, Menter T, et al. Practical aspects of COVID 19 autopsies. Pathologe. (2021) 42:197–207. doi: 10.1007/s00292-021-00925-w

# DISCUSSION

The COVID 19 pandemic had a significant impact on the Italian National Health Service (NHS). During the initial wave of the pandemic, the Italian NHS had to reorganize a large portion of its resources towards handling patients with SARS-CoV-2 infection and implementing specific protocols of action (SPA) to prevent the spread of the infection among healthcare professionals and patients. Initially, autopsies were only performed occasionally in a few hospitals with BSL3 autopsy rooms, which were part of the national network for autopsies in patients with high-risk infectious diseases like Creutzfeldt-Jakob disease.

The majority of published articles have focused on various aspects, clarifying the clinical presentation, diagnostic tests, treatment modalities, and hospitalization management associated with COVID-19. However, there is a lack of thorough characterization when it comes to pathological and laboratory issues, such as autopsy procedures and cadaver handling. As a result, several governments have enacted strict policies regarding the management of corpses of individuals who died from or with COVID-19, raising significant ethical questions. These policies have been implemented without any individualized risk assessment, as a precautionary measure to minimize infectious hazards.

For instance, in Italy, the initial European country to experience the COVID-19 outbreak, burial procedures were immediately prohibited based on the first published studies regarding the virus's environmental stability. Initially, cremation was promoted for deaths related to COVID-19. The visitation of the deceased's body and funeral ceremonies were also forbidden, and any prayers during the closing of the coffin were halted. Additionally, burial with personal belongings and clothing was prohibited. To minimize the time between death and cremation, the deceased was directly taken to the cemetery where a short burial ceremony was conducted. The Autopsy Work Group of the Spanish Society of Anatomical Pathology and the Royal College of Pathologists also advised against performing autopsies on positive COVID-19 cases, offering similar recommendations.

he guidance of World Health Organization (WHO) in its document has suggested that, apart from cases of hemorrhagic fevers and cholera, the corpse of a subject who died from/with COVID-19 should generally be considered non-infectious. These indications, however, were more stringent compared to WHO's recommendations. WHO has emphasized the need for careful lung management during autopsies. Unfortunately, the restrictions on autopsies during the COVID-19 pandemic have hindered the acquisition of data on the new virus. Early autopsies revealed that

SARS-CoV-2 not only causes respiratory disease but also affects other vital organs. This highlights the importance of "learning from death." Although several studies have examined the risks of SARS-CoV-2 infection for those handling, transporting, and examining deceased individuals with COVID-19, to our knowledge, no research has investigated the vitality of the virus in post-mortem samples.

With regard to these considerations, the objective of this experiment was to determine whether and for how long SARS-CoV-2 remains capable of replicating in the tissues of individuals who have died from or with COVID-19, posing a tangible risk of infection.

The uniqueness of this experimental study lies in its groundbreaking revelation that viral replication ceases after 24 hours in samples obtained from deceased individuals with COVID-19. It is important to note that this timeframe could potentially be shorter, as only one sample tested positive in the case of an autopsy conducted 12 hours postmortem, with the body stored at 0 ◦C. Several studies have identified the presence of SARS-CoV-2 viral RNA in cadavers, yet it is widely acknowledged that the detection of viral RNA does not necessarily indicate infectiousness. To date, SARS-CoV-2 has been detected in various post-mortem samples, including swabs taken from the eyes, nose, and mouth, periodontal tissue, respiratory tract (such as the nasopharynx, throat, and lungs), and other tissues and bodily fluids. No studies have demonstrated transmission of SARS-CoV-2 from a deceased individual to a living subject, and there have been no reports of infections occurring during autopsies of SARS, MERS, or COVID-19 cases. This contrasts with the Ebola virus, which is known to be transmitted through contact with deceased bodies. In a study by Prescott et al.[98], the authors performed research using an animal model (cynomolgus macaques), the viable virus was isolated <7 days post euthanasia; contrariwise, viral RNA was detectable for 10 weeks. It is important to note that the Ebola virus is an RNA virus from the Filoviridae family, while SARS-CoV-2 is an RNA virus from the Coronaviridae family.[99,100,101] In a recent report, the CDC summarized the suggestions in order to manage he corpse of subjects who had died infected by SARS-CoV-2 or Ebola viruses[102].

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Up until now, numerous autopsies have been carried out on individuals who passed away as a result of or alongside COVID-19. The lack of reports of a direct correlation between infection and post-mortem investigation suggests that autopsies should be considered a safe procedure, particularly when all recommendations are followed. The surge in deaths worldwide due to the COVID-19 pandemic has increased the risk associated with moving corpses, necessitating a greater awareness of standard precautions, applying good practice, and guidelines. Given that SARS-CoV-2 was an unknown etiological pathogen, a prudent approach was taken. Viral cultures for infectious COVID-19 represent the best way to determine virus viability and infectivity, and the experimental results suggest that the risks of SARS-CoV-2 transmission may be considered minimal in handling, transporting, and examining deceased individuals with COVID-19. In particular, considering the data provided by this study and recent literature, it may be confirmed that the risk of cadaveric infection in individuals who died from or with COVID-19 is extremely low and related to the first hours after death, becoming very low after 12 hours. These findings are also important for non-healthcare professionals, such as funeral directors or morticians.

To date, there is no scientific evidence of a higher incidence of COVID 19 infection or mortality among these occupational groups, although they were considered as high-risk categories. These data suggest that risk reduction measures are being successfully applied. Moreover, as reported in the ad interim guidance of WHO titled “Infection Prevention and Control for the Safe Management of a Dead Body in the Context of COVID 19”, it may be confirmed that cadavers do not transmit the infection at a PMI no less of 12 h.[103] In accordance with the current findings, challenging questions will arise. For instance, was a complete prohibition on funerals necessary, or could families still bid their "final farewell" despite the physical distance? Within European nations, honoring cultural and religious customs, along with the dignity of the deceased, must always be upheld and safeguarded. In an exceptional and unforeseen circumstance like the COVID-19 pandemic, numerous difficulties arose in managing these sensitive aspects, resulting in families being deprived for the first time of the painful opportunity to bid farewell to their loved ones. Undoubtedly, there has been a lack of scientific evidence, which, through focused studies, could have allowed for a rational management of the issue. For instance, various governments implemented stringent constraints on the handling of individuals who have died from/with COVID-19, despite the WHO's suggestion that proper use of personal protective equipment (PPE) was sufficient to ensure the dignity of the deceased and the respect for cultural and religious

[103] WHO Interm Guidance Infection Prevention and Control for the safe management of a dead body in the context of COVID-19. J. Hosp. Infect. 2020, 104, 246–25

customs.[103]. Moreover, the results of the present study support the effectiveness of adherence to international guidelines and/or recommendations during the post-mortem investigation on infection control among the pathology staff (technicians, biologists, pathologists). For these reasons, we disagree with the commentary of Sapino et al.[104], where the authors reported that autopsies should be restricted to well-motivated cases. In contrast, we emphasize the significance of autopsies, particularly in the management of unknown diseases. Following the autopsy, the staff involved in carrying out these complete autopsies underwent nasopharyngeal swabs for SARS-CoV-2 and tested negative (the autopsies involved an exposure time for the medical and technical staff of 2 hours). It's essential to note that this study was conducted on COVID-19 corpses during autopsy. This represents the strength of this study as it allowed us to evaluate environmental contamination during a COVID-19 autopsy. The main limitation of this study is that we detected the positivity of swab samples collected during autopsy procedures through real-time PCR without assessing the risk of causing an infection. This limitation has been reported in several previous studies, and it's imperative to stress that before sending alarming messages, the forensic community must fully comprehend the weight of the evidence.

Our experience relating to the safe performance of autopsies suggests that: 1) the initial alarmism was completely unjustified; 2) autopsies remain the gold standard for understanding the pathophysiological alterations that affect the body, representing an indispensable tool not only for diagnostic purposes but also for refining health treatments in relation to the target organs involved.

Indeed, as it has been widely demonstrated that COVID 19 is a systemic disease[105], in which viral RNA is still present several days after death, most frequently in the respiratory tract and associated with severe and fatal organ damage[106]. However, the exact pathophysiology behind organ damage and the time after death while virus could still replicate remain unclear.

Whole-body autopsies are an essential tool for determining the extent of organ involvement and consequently, for obtaining a more accurate diagnosis. Furthermore, they should be considered mandatory to define the exact cause of death, which would provide useful clinical and epidemiologic information, as well as pathophysiological insights to further provide therapeutic tools[107]. Regrettably, due to several issues of infection control and for logistical and operational

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reasons, as previously mentioned, full-body autopsy studies of COVID 19 have been limited and, as with lung autopsy studies, often include few patients.[108,109]

According to scientific literature, the main findings in COVID 19 patients are:

**Lungs**

The main lung histological findings reported are represented by heavy, edematous and

opportunity and the lockdown of science. J Clin Med. (2020) 9:1472. doi: 10.3390/jcm9051472

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congested lungs[110,111,112,113,114,115,116,117,118,119,120,121,122,123,124,125,126,127,128,129,130,131,132,133] with

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reddish-dark areas.[107,115,117,119,120,122,124,131,134] Pulmonary consolidations of different sizes, from patchy to diffuse, were also described.[110,112,119,127,132,133,135,136] In some cases, clear hemorrhagic areas[112,113,119,121] and whitish fibrotic areas[115] were reported. Some groups of researchers described thromboemboli in large pulmonary arteries and/or small and mid-sized arteries.[113,134,137,138,139] In some cases, pleura showed signs of pleurisy with pleural adhesion and effusion. [110,112,117,119,127,133, 134,135,140,

The most encountered histological finding was diffuse alveolar damage (DAD) at different stages, mainly in exudative and proliferative phases, characterized by hyaline membranes, intra-alveolar and/or interstitial edema also proteinaceus, intra-alveolar fibrinous exudate, intra-alveolar mononuclear cells infiltrates (macrophages, lymphocytes, neutrophils), type 2 pneumocyte hyperplasia/activation, squamous

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metaplasia.[126,131,120,112,110,128,115,117,130,125,133,121,132,111,124,119,113,127,118,106,134,135,136,141,138,137,140,142,143,]

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144,145,146,147,148,149,150,151,152,153,154,155,156,157,158,159,160

In a few cases, alveolar hemorrhage was observed.[113,116,153,138,121,126,130] Pneumonia or bronchopneumonia pictures were also described as focal or diffuse.[126,115,117,124,122,113,134,135,141,150,111,159] In several cases, the presence of fibrin-enriched thrombi in vessels was reported, mostly appearing as microthrombi in alveolar capillaries and/or

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in small vessels.[111,156,112,113,149,114,153,118,119,138,160,124,125,141,126,127,159,134,135,136,151,139,130,131,142]

Aiolfi et al.[142] found massive intravascular hemorrhagic thrombosis of peripheral vessels associated with diffused endothelial hyperplasia and general thickening of the muscular wall.[142] Moreover, damage of small vessels was reported as thrombotic necrotizing capillary injury[123], infiltrate of lymphocytes and plasma cells[117], vasculitis[124,127], septal capillary damage[149], endothelial tumefaction with a large number of pulmonary megakaryocyte in capillaries[153], perivascular inflammation/ endothelialitis[152,158,161]. Additionally, Ackermann et al. described fibrin thrombi in arterioles associated with intussusceptive angiogenesis.[152]

Several immunohistochemical markers were used to identify better the inflammatory cells infiltrates. T and B cells were investigated by CD3, CD5, CD4 (T helper cells), CD8 (cytotoxic T cells) and CD20 (B lymphocytes) antibodies;[120,110,115,114,124,119,113,127,141,138,137,142,150,152,156,158,159,161] a study reported the analysis of CD57 + showing the presence of sparse Natural Killer cells not varying according to DAD pattern.[141] Macrophages, analyzed using CD68, were mostly sited in alveolar spaces and in fibroproliferative areas.[152,110,156,113,114,115,119,138,124,125,141,130] CD61 was used to analyze thromboemboli.[152,137,113,114,159]

Magro et al.[123] analyzed the complement components C4d and C3d, the terminal complex C5b-9 or membrane attack complex (MAC), and MASP-2 observing the deposition of MAC within the lung septal microvasculature also in normal-appearing lung. As for the routine histology data on type II pneumocytes, TTF-1 (thyroid transcription factor-1) was used to evaluate the involvement of such cells, which appeared, in some cases, enlarged, hyperplastic and atypical with nucleoli viral cytopathic-like changes and many mitotic figures.[142,113,114,119,124,125,141,136,151,130] Angiotensin-converting-enzyme-2 (ACE-2) was investigated as a receptor for host cell entry of SARS- CoV-2, found positive in alveolar epithelial, endothelial cells, alveolar macrophages, and lymphocytes in lung tissue samples.[152,115,138] Some researchers carried out immunohistochemistry for virus detection using specific antibodies for nucleocapsid protein (NP) or spike and envelope proteins of SARS-CoV-2, whose positivity was observed in pneumocytes, alveolar macrophages, intralveolar septa, and septal capillary[112,123,143,159,134,136,157] reported negative immunostaining in two cases out of total of seven.

Some authors performed the molecular diagnosis of COVID 19 infection using RT-PCR performed by nasopharyngeal, oropharyngeal or tracheobronchial swabs[110,111,113,115,117,140,121,124,148,129], or analyzing lung tissue sampled during

[161] Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID 19. The Lancet 395 (10234), 1417–1418. doi:10.1016/S0140-6736(20)30937-5

autopsy[112,145,138,120,160,121,143,141,127,131]

Finally, the lung damage was investigated by electron microscopy showing viral particles predominantly located in pneumocytes[112,114,119,132], free in alveolar space[132], in phagosomes of alveolar macrophages[143], confirming the above reported immunohistochemical findings; moreover, the viral particles were observed either outside or inside the cells in aggregates confined within vesicles.[112] Interestingly, Ackermann et al.[152] described distorted lung vascularity with structurally deformed capillaries which appeared elongated, with changes in caliber and intussusceptive pillars, and endothelium ultrastructural damage.

**Heart**

The gross examination of the heart showed myocardial ventricular hypertrophy and dilatation, mainly of the right cavity, in a considerable number of cases.[137,113,119,122,124,127,133] Acute right coronary artery thrombosis was observed in one case.[137] The most frequent microscopic findings included cardiomyocyte hypertrophy[158,121,122,124,141,132], myocardial fibrosis[116,158,162,122,141,134], focal lymphocytic infiltrate[113,122,163,127,159,148], individual cardiomyocyte injury[113,163], interstitial edema[158,163,141,127,132], acute or previous myocardial infarction[124,141,134], coronary artery atheroma and/or atherosclerosis.[121,133] Other rare but significant histopathological changes included amyloidosis[122,124], coronary small vessel disease[122], fibrin microthrombi[137,141,127], thrombosis of myocardial veins[122,159], endocardial thrombi in the left ventricle[122], lymphocytic myocarditis/epicarditis/ pericarditis.[137,113,159] Moreover, Tavazzi et al.[164] examined samples of cardiac tissue using TEM, which revealed the presence of a small group of viral particles or single particles within the damaged interstitial cells of the myocardium, also showed loss of plasmalemma integrity. Lindner et al.[163] clearly described the presence of SARS-CoV-2 RNA in interstitial cells, and macrophage infiltrates by in situ hybridization (ISH) performed on paraffin-embedded left ventricle samples. The gross examination of the liver showed signs of steatosis[122,124] as the most frequent finding, while in some cases, signs of shock necrosis[124] were observed. In one case, a macroscopic infarction was detected.[137] The most frequent microscopic

[162] Escher, F., Pietsch, H., Aleshcheva, G., Bock, T., Baumeier, C., Elsaesser, A., et al. (2020). Detection of viral SARS-CoV-2 genomes and histopathological changes in endomyocardial biopsies. ESC Heart Fail. 7, 2440. doi:10.1002/ehf2.12805

[163] Lindner, D., Fitzek, A., Bräuninger, H., Aleshcheva, G., Edler, C., Meissner, K., et al. (2020). Association of cardiac infection with SARS-CoV-2 in confirmed COVID 19 autopsy cases. JAMA Cardiol. 5, 1281–e203551. doi:10.1001/jamacardio.2020.3551

[164] Tavazzi, G., Pellegrini, C., Maurelli, M., Belliato, M., Sciutti, F., Bottazzi, A., et al. (2020). Myocardial localization of coronavirus in COVID 19 cardiogenic shock. Eur. J. Heart Fail. 22 (5), 911–915. doi:10.1002/ejhf.1828

findings included steatosis[158,154,121,122,141,127,155,134,165] , chronic congestion[121,122,141,155], lymphocytic infiltrates especially in the portal/periportal tract[154,122,134,148,161,165], hepatocyte necrosis[158,122,141,161,165], hyperplasia, and hypertrophy of the Kupffer cells[122,141,155]. Less reported findings were central lobular pallor[121], cholestasis, and ductular proliferation[122], focal lobular inflammation with predominant lymphocytes.[140,165] Rapkiewicz et al.[159] observed platelet-fibrin microthrombi in hepatic sinusoids and larger platelet aggregates in the portal veins. Sonzogni et al.[165] reported variable degrees of portal vein endotheliitis, diffuse alterations of intrahepatic vascular structures (portal branches and sinusoids) and variable degrees of partial/ complete luminal thrombosis. Wang et al.[140] studied the hepatocyte ultrastructural morphology in two different liver samples, revealing the presence of typical coronavirus particles in the cytoplasm mostly without membrane-bound vesicles.

**Kidneys**

The gross examination of kidneys did not reveal any particular finding. The most frequent and relevant microscopic evidence included acute tubular damage[137,158,166,122,124,127,167,128,132] and fibrin microthrombi in glomeruli.[137,141,127,159,167] Yan et al.[132] described a focal acute tubular injury with flattened epithelium and lumens containing sloughed epithelial lining cells, granular casts, Tamm-Horsfall protein, and intraluminal accumulation of cellular debris in focal areas. Other less frequent changes were disseminated intravascular coagulation[124], hemosiderin in renal tubules[134], chronic interstitial inflammation with sporadic prominent perivascular lymphocytic inflammation[112], hypertensive and diabetic nephropathy[124], and unspecific nephrosclerosis[122]. Other researchers also performed TEM, which revealed prominent activation of podocytes with multiple cytoplasmic vesicles containing virus-like particles, also detected in endothelial cells and proximal tubular epithelial cells.[112,124] The virions were also detected in proximal convoluted tubules.[159]

**Other organs**

Data on brain involvement in COVID-19 are controversial. In particular, in a study conducted on six autopsy cases, von Weyhern[168] et al. observed massive intracranial hemorrhage and diffuse

[165] Sonzogni, A., Previtali, G., Seghezzi, M., Grazia Alessio, M., Gianatti, A., Licini, L., et al. (2020). Liver histopathology in severe COVID 19 respiratory failure is suggestive of vascular alterations. Liver Int. 40 (9), 2110–2116. doi:10.1111/liv.14601

[166] Kudose, S., Batal, I., Santoriello, D., Xu, K., Barasch, J., Peleg, Y., et al. (2020). Kidney biopsy findings in patients with COVID 19. Jasn 31 (9), 1959–1968. doi:10.1681/ASN.2020060802

[167] Santoriello, D., Khairallah, P., Bomback, A. S., Xu, K., Kudose, S., Batal, I., et al. (2020). Postmortem kidney pathology findings in patients with COVID 19. Jasn 31 (9), 2158–2167. doi:10.1681/ASN.2020050744

[168] von Weyhern, C. H., Kaufmann, I., Neff, F., and Kremer, M. (2020). Early evidence of pronounced brain involvement in

petechial hemorrhages along with microscopic findings of localized perivascular and interstitial encephalitis, neuronal cell loss, and axon degeneration of multiple neuronal areas. Remmelink et al.[134] described cerebral focal necrosis and cerebral hemorrhage. Similarly, another study[169] on a single case described destructive hemorrhagic white matter lesions, focal microscopic necrosis, perivascular cellular infiltrates, and axonal injury, then confirmed by the immunohistochemical positivity to different markers such as CD68, CD3, CD20, GFAP (glial fibrillary acidic protein), APP (amyloid precursor protein) and PLP (myelin proteolipid protein). Conklin et al.[170] reported microscopic ischemic lesions associated with widespread microvascular injuries as perivascular and parenchymal petechial hemorrhages. The ischemic damage was also found in another report by the BAPP (β amyloid precursor protein) immunohistochemical stain and T-cell infiltration around blood vessels and capillaries.[137]Similar findings were described by Kantonen et al.[171], reporting enlarged perivascular spaces, microhemorrhages, scattered T-lymphocytes, and minor intravascular fibrinoid deposits in some cerebral and subarachnoidal vessels.Moreover, in the autopsy cases examined by Duarte-Neto et al.[141], reactive gliosis, neuronal satellitosis, small vessel disease, and perivascular hemorrhages were reported. On the contrary, Solomon et al.[172] described only acute hypoxic-ischemic damage in the absence of microscopic specific elements; however, the same research group highlighted negative SARS-Cov-2 immunohistochemistry and positive molecular diagnosis by RT-PCR in few samples of the medulla, olfactory nerves, and frontal lobe.

Some reports described macroscopic and histological evidence of the spleen. Oprinca and Muja[127] reported histologically marked congestion and white pulp atrophy associated with the absence of lymphoid follicles. Prilutskiy et al.[155] observed an enlarged, soft, and friable organ just in one of the four analyzed cases. Microscopically, they described white pulp depletion with red pulp hemorrhage or infarction and histiocytic hyperplasia with hemosiderin-laden macrophages, suggestive of a prior red blood cells phagocytosis, or hyperplastic white pulp with red pulp congestion but lacking hemophagocytosis. White pulp depletion and red pulp hemorrhage were

fatal COVID 19 outcomes. The Lancet 395 (10241), e109. doi:10.1016/S0140-6736(20)31282-4

[169] Reichard, R. R., Kashani, K. B., Boire, N. A., Constantopoulos, E., Guo, Y., and Lucchinetti, C. F. (2020). Neuropathology of COVID 19: a spectrum of vascular and acute disseminated encephalomyelitis (ADEM)-like pathology. Acta Neuropathol. 140 (1), 1–6. doi:10.1007/s00401-020-02166-2

[170] Conklin, J., Frosch, M. P., Mukerji, S., Rapalino, O., Maher, M., Schaefer, P. W., et al. (2020). Cerebral microvascular injury in severe COVID 19. medRxiv. doi:10.1101/2020.07.21.20159376

[171] Kantonen, J., Mahzabin, S., Mäyränpää, M. I., Tynninen, O., Paetau, A., Andersson, N., et al. (2020). Neuropathologic features of four autopsied COVID 19 patients. Brain Pathol. 30, 1012. doi:10.1111/bpa.1288910.1111/ bpa.12889

[172] Solomon, I. H., Normandin, E., Bhattacharyya, S., Mukerji, S. S., Keller, K., Ali, A. S., et al. (2020). Neuropathological features of COVID 19. N. Engl. J. Med. 383 (10), 989–992. doi:10.1056/NEJMc2019373

reported also by Rapkiewicz et al.[159] Even Nunes Duarte-Neto et al.[141] studied the spleen in five cases reporting lymphoid hypoplasia, red pulp hemorrhages, and splenitis. Additionally, they described follicular arterioles endothelial changes, vasculitis, and arterial thrombus. Likewise, an acute splenitis was observed by Menter et al.[124] in six out of 21 cases, while Lax et al.[122] found lymphocyte depletion affecting both the spleen and lymph nodes. Furthermore, in two cases necrotizing granulomata was reported[128] in the spleen.

Lymph nodes and bone marrow histological changes have been observed in two of the above-mentioned studies. Indeed, one of these[155] described enlarged mediastinal and pulmonary lymph nodes that showed a hemophagocytic histiocytes CD163+, while the other[124] reported lymph nodes congestion and increased presence of plasmablasts. As for the bone marrow, both research groups reported left- shifted myeloid hyperplasia; in addition, Prilutskiy et al.[155], also observed histiocytic cells CD163+. Wang X.-X. et al.[158] reported data on lymphoid tissue describing primary lymphoid follicle, scattered T lymphocytes, and focal necrosis. The virions detection in bone marrow was reported only in one study using TEM, which detected megakaryocytes.[159]

Other interesting evidence was provided by Varga et al.[161], who described mesenteric ischemia and small bowel sub-mucosal vessels endotheliitis. One case of ischemic enteritis was also reported.[134] Ischemic bowel changes were observed by Skok et al.[139], namely atrophic cripts, cryptitis, ulceration, and hemorrhage. Some cases of pancreatitis were also detected.[137,122]

Adrenal gland findings were reported by Iuga et al.[173], who described small vessels with acute fibrinoid necrosis, subendothelial vacuolization, and apoptotic debris. In a further study[122], adrenal cortical hyperplasia was described. Interestingly, Hanley et al.[137] described patchy areas of infarct-type adrenocortical necrosis and organizing microthrombi in adrenal vessels.

Furthermore, Yang et al.[174] studied the tests in 12 cases using post-mortem biopsy, detecting Sertoli cells swelling, reduced Leydig cells, mild lymphocytic inflammation, detachment from tubular basement membranes and lumen intratubular cell mass loss and sloughing; in the same study, the immunohistochemical positivity to different markers such as CD3, CD20, CD68, CD138, and ACE-2 was observed, but the RT-PCR confirmed the presence of the virus only in a biopsy sample. Nevertheless, Nunes Duarte-Neto et al.[141], in two cases out of two, observed an orchitis condition.

[173] Iuga, A. C., Marboe, C. C., Yilmaz, M. M., Lefkowitch, J. H., Gauran, C., and Lagana, S. M. (2020). Adrenal vascular changes in COVID 19 autopsies. Arch. Pathol. Lab. Med. doi:10.5858/arpa.2020-0248-LE

[174] Yang, M., Chen, S., Huang, B., Zhong, J.-M., Su, H., Chen, Y.-J., et al. (2020). Pathological findings in the testes of COVID 19 patients: clinical implications. Eur. Urol. Focus 6 (5), 1124–1129. doi:10.1016/j.euf.2020.05.009

Finally, the involvement of the skin was included in the study performed by Magro et al.[123]. Five cases with purpuric lesions were described, microscopically characterized by thrombogenic vasculopathy, epidermis, and adnexal structures necrosis, interstitial and perivascular neutrophilia with prominent leukocytoclasia or perivascular lymphocytic infiltrate in the superficial dermis with small thrombi within rare venules of the deep dermis. The same study highlighted the immunohistochemical positivity to different markers like C4d, C3d, C5b-9, MASP2, and SARS-CoV-2 spike and envelope proteins. Purpuric lesions, superficial perivascular mononuclear infiltrate, and endothelial changes were also described by another work in which interesting findings in the skeletal muscle were evaluated, consisting of myositis and necrotic fibers.[141]

In our study, a median of 11 organs per necropsy presented histopathological findings, confirming the multisystemic nature of COVID-19. Thus, the existence of pathological findings in heart or kidney was associated with a high proportion of pathological findings in lung, liver, lymph nodes and CNS in most patients. Similarly, the presence of hemaphagocytosis in spleen, bone marrow and lymph nodes were suggestive of a systemic disease.

Another interesting topic of debate is related to the ability of NHS to manage a probably future pandemic infection. In particular, are we ready for another pandemic? The pandemic caught us unprepared, but now that we have 3 years of experience, would we be able to face a new infection in the future? Certainly not, because all the measures put in place to strengthen the National Health System, now that the pandemic is over, have been dismantled or reduced, diverting economic resources towards other objectives. The need to have a pandemic plan should be, in the modern era, a priority. During the COVID 19 pandemic, we realized that the virus' ability to mutate represents an important challenge for the scientific community. Mutations can compromise both the subject's active defenses and the ability of healthcare professionals to treat the disease. It becomes necessary to predict and prevent any infections mediated by COVID variants, or by other pathogens, in order to avoid a national lockdown, with dramatic implications both on the nation's economy and on the psycho-physical well-being of its citizens.

# CONCLUSION

The strengths of this current project lie in the autopsy reports, which serve as a foundational basis for comprehending the consequences of infection. Firstly, they demonstrate that post-mortem investigations, such as gross examination, histological and immunohistochemical analysis, electron microscopy, and molecular testing, are indispensable tools for various reasons. Through these investigations, it is possible to distinguish whether the subject died 'from' or 'with' COVID-19, providing reliable epidemiological, pathological, molecular, and global health data. However, our study has limitations, primarily related to the number of patients recruited and the lack of information regarding the prevalence of detected features stratified by characteristics such as age, gender, race, and comorbidities, as well as their pharmacological treatment. Nonetheless, all post-mortem findings, along with clinical pictures, are crucial for better understanding pathogenesis and pathophysiology, ultimately leading to the most effective therapeutic management of patients. Therefore, this is the right time “to reverse the decline of autopsy rates”, as affirmed by De Cock et al.[175] a few months before the COVID-19 outbreak, in order to benefit global public health. As observed by Barth et al.[176], a “call to action” for more autopsy reports containing detailed findings is necessary.

[175] De Cock, K. M., Zielinski-Gutiérrez, E., and Lucas, S. B. (2019). Learning from the dead. N. Engl. J. Med. 381 (20), 1889–1891. doi:10.1056/NEJMp1909017

[176] Barth, R. F., Xu, X., and Buja, L. M. (2020b). A call to action. Chest 158 (1), 43–44. doi:10.1016/j.chest.2020.03.060