Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

The histological and immunopathological landscape of lung autopsy sample of COVID 19

Lana Sardar Saleh*

Department of Biology, College of Science, Salahaddin University- Erbil, Kurdistan Region- Iraq

ARTICLE INFO

Original paper

Article history:
Received: February 07, 2022
Accepted: April 13, 2023
Published: April 30, 2023

Keywords:

COVID19, SARS-CoV-2, pathology, immunopathology, complication

ABSTRACT

The purpose of this study is to explain the reason for the death histologically by comparing normal lungs with infected ones. The lung autopsy samples were taken from 12 adult patients in Erbil forensic medicine diagnosed before with covid 19 which also consider a reason for death. For histological examinations and the identification of SARS-CoV-2 RNA, autopsy materials were collected, fixed in 4% neutral formaldehyde for at least 24 hours, and sampled as formalin-fixed, paraffin-embedded (FFPE) tissues. Staining with hematoxylin and eosin (H&E) was done in accordance with protocol. Based on the results of immunopathology in deceased people, it was shown that there was a strong positive reaction with BCL2 antibody in the cytoplasm of lung alveolar cells compared to the lungs of healthy people. Also, a positive reaction with catenin antibody, SMA antibody had occurred in the cytoplasm of lung alveolar cells in the lungs of patients, and finally, it was shown that the Vimentin antibody reaction was present in the cytoplasm of lung alveolar cells of patients. All four investigated factors, BCL2, catenin, SMA antibody and vimentin antibody, have played an important role in the inflammation and fibrosis of lung tissue in patients with COVID, and the combination of these four factors together has played a significant role in worsening the symptoms and worsening of the disease.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.4.11

Introduction

SARS-CoV-2 is the coronavirus that causes COVID-19 (1). Coronavirus disease 2019 (2) was initially reported in December 2019 in Wuhan, China, among a group of people who had pneumonia of unclear cause (3). In March 2020, COVID-19 sparked the fifth-worst epidemic in recorded human history. More than 474 million individuals have been infected with COVID-19, and 6.1 million have died as a result (4). The positive-sense, single-stranded, big RNA virus coronaviruses are enclosed and infect a variety of species, including humans. Since they have a "crown-like" shape thanks to their spherical morphology, core-shell, and glycoprotein projections from their envelope, they are known as coronaviruses (5). They contain the biggest RNA viral genomes, with average sizes between 27 and 32 kB. The nucleocapsid protein (N), the spike protein (S), the small membrane protein (SM), and the membrane glycoprotein (M) are all encoded by their four primary structural genes (6). One well-known feature of coronavirus disease (2) is hyperinflammation, which has been linked to organ failure, illness severity, and mortality (7,8). The most common cause of fatal COVID-19 is critical oxygenation impairment, and corticosteroid therapy has been shown to significantly lower mortality in these cases (2,9,10). An increasing body of research suggests that the severity of COVID-19 is influenced by an inflammatory condition brought on by the immune system's hyperactivation8,12 in an effort to eradicate the virus. Chronic inflammation can harm lung tissue, cause pulmonary-edema fluid to exude, and cause dyspnea and acute respiratory distress syndrome (11–13). Da Silva SR et al (2021), proved in their study that, broad SARS-CoV-2 cell tropisms, significant infiltration of innate immune cells, activation and depletion of adaptive immune cells, and severe tissue destruction, thromboembolic, excessive inflammation, and weakened immune responses are all present in deadly COVID-19 lungs (14). In their study, Hans Bösmüller et al (2021), emphasized that tissue-based examination can help them significantly with the histopathological examination of tissues and a better understanding of pathophysiology (15). Since the majority of COVID-19 histological changes are detected in blood vessels and the lungs, they appear to have the most clinical significance (16). So, clinicians can significantly enhance disease therapy and prognosis by having a thorough grasp of the underlying immunopathological processes of COVID-19 and its histopathological characteristics. Therefore, the purpose of this study is to explain the reason for the death histologically by comparing normal lungs with infected ones.

Materials and Methods

Study participants and ethical issues

The lung autopsy samples were taken from 12 adult patients in Erbil forensic medicine diagnosed before with covid 19 which also consider a reason for death. All deceased people satisfied the COVID-19 diagnostic requirements, and polymerase chain reaction (PCR) studies on all autopsy cases verified the presence of SARS-CoV-2. Basic patient information and clinical data were obtained from electronic medical records from hospitals and foren-

* Corresponding author. Email: Lana.alalem@su.edu.krd

sic medicine.

This study was approved by the ethics committee of Salahaddin University-Erbil and was conducted with the written consent of the patient's family members in accordance with the regulations issued by the Iraqi National Health Commission and the Declaration of Helsinki.

Histology

For histological examinations and the identification of SARS-CoV-2 RNA, autopsy materials were collected, fixed in 4% neutral formaldehyde for at least 24 hours, and sampled as formalin-fixed, paraffin-embedded (FFPE) tissues. Staining with hematoxylin and eosin (H&E) was done in accordance with protocol. To examine the collagen and fibrin fibers in lung tissues, Masson staining was used. Using the streptavidin-biotin-peroxidase method and diaminobenzidine, immunohistochemical staining (IHC) was carried out. Each antibody clearly depicts a genetic, cytological, or histological characteristic found inside the cells. For instance, Bcl2 is a crucial regulator of apoptosis, which either opposes or promotes cell death. So, using this dye illuminates what transpires within the tissue.

Results

Socio-demographic characteristics

In this study, 12 patients who died due to COVID-19 were compared with 12 healthy individuals (without COVID-19 disease) in terms of lung histology and immunopathology. The results of examining demographic variables in patients who died due to COVID-19 showed that their mean age was 68 years and the mean age of healthy patients was 66 years. The sex of the patients in the COVID-19 group was as follows: 8 were male and 4 were female. While in the group of healthy people, there were 7 men and 5 women. The time interval between the appearance of symptoms and death in COVID-19 patients was 9.8 (0-23) days on the mean. Cardiovascular disease was the most common co-morbidity in the COVID-19 group Hypertension, diabetes and COPD were also seen in this group. Cardiovascular disease was the most common disease in the group of healthy people. The most common clinical symptoms based on the data available in the medical records of the patients were fever and cough In addition, shortness of breath and kidney failure were also seen in these patients. The mean hemoglobin in the patients of the COVID-19 group was 12.8 (gr/dL). Other information is shown in Table 1.

Histological and immunopathological

Lung immunopathology status was investigated based on BCL2 antibody, Catenin antibody, SMA antibody and Vimentin antibody in two groups of COVID-19 patients and healthy individuals. The results of examining BCL2

Table 1. Clinical data of all patients.

Characteristics	N	% or range
COVID-19 group (12)		
Age	68 y	54 – 84 y
Gender	8 male / 4 female	12
Hospitalizations	7.6 d	0- 18 d
Time between symptoms and death	9.8 d	0-23 d
Comorbidities		
Hypertension	8	67%
Diabetes	5	42%
Cardiovascular disease	9	75%
COPD	4	33%
Initial clinical presentation		
Cough	11	92%
Fever	12	100%
Dyspnea	7	58%
Renal failure	6	50%
Laboratory results		
WBC (109 /L)	7.92±1.64	7.12 – 8.24
Hemoglobin(gr/dL)	12.8	10.8 - 14.1
PLT (109 /L)	201.44±87.9	199.45 – 204.54
Cr (mg/dl)	2.1±0.4	1.6 – 2.7
ESR (mm/hr)	55.84±22.61	51.4 - 60.5
CRP (mg/L)	56.5±28.7	53.64 - 59.23
Non COVID- 19 group		
Age	66 y	53 – 81 y
Gender	7 male / 5 female	12
Comorbidities		
Hypertension	5	42%
Diabetes	5	42%
Cardiovascular disease	6	50%
COPD	4	33%

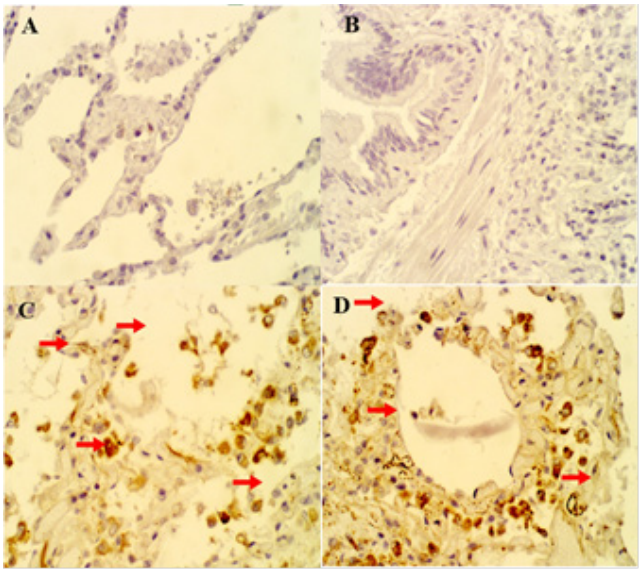

Figure 1. Normal lung group. (A) (B). Showed negative reaction with BCL2 antibody in the walls of alveoli. IHC-BCL2-DAB stain. 400x. **COVID-lung group. (C) (D).** Showed a strong positive reaction with BCL2 antibody in the cytoplasm of alveolar cells of the lung (arrow) which appear as golden-brown cytoplasmic granules. IHC-BCL2-DAB stain. 400x.

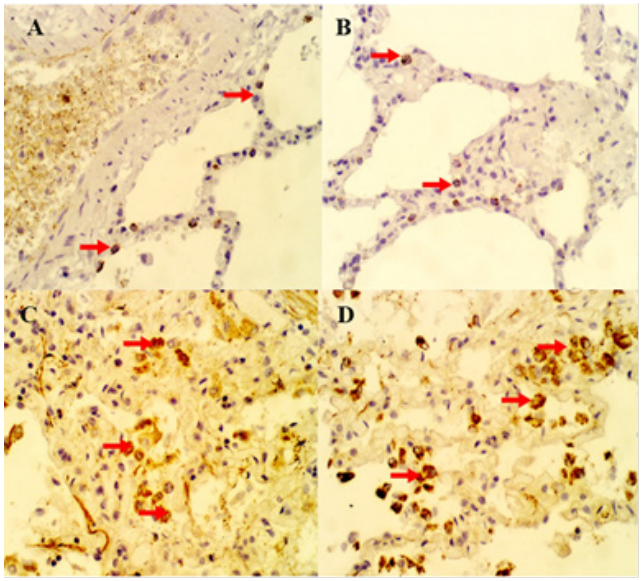

Figure 2. Normal lung group. (A) (B). Showed a weak positive reaction with the Catenin antibody in the alveolar cells of the lung which appear as golden-brown granules in the cytoplasm (arrow). IHC-Catenin-DAB stain. 400x. **COVID-lung group. (C) (D).** Showed a strong positive reaction with *Catenin* antibody in the cytoplasm of alveolar cells of the lung (arrow) which appear as golden-brown cytoplasmic granules. IHC-*Catenin*-DAB stain. 400x.

antibody in the lungs of healthy people showed that there is a negative reaction with BCL2 antibody in the alveolar wall (Figure 1 (A) (B)). While in the lungs of the group of COVID-19 patients, there was a strong positive reaction with BCL2 antibody in the cytoplasm of alveolar cells of the lung, which appeared as golden-brown cytoplasmic granules. This reaction occurs in COVID-19 patients due to the disruption in the apoptosis process, it provides a faster process of cell death and tissue destruction Fig 1 (C), (D).

The results of the Catenin antibody examination in the lungs of healthy people showed that there was a weak positive reaction with Catenin antibody in the alveolar cells of the lung, which appeared as golden brown granules in the cytoplasm (Figure 2 (A) (B)). While in the lungs of the group of COVID-19 patients, there was a strong positive reaction with catenin antibody in the cytoplasm of lung alveolar cells, which appeared as golden-brown cytoplasmic granules. Catenin is a central component of the Wnt signaling pathway, which plays a key role in regulating cell proliferation, differentiation and apoptosis. It seems that this positive reaction has an important effect on the process of tissue destruction in COVID-19 patients (Figure 2 (C) (D)).

The results of SMA antibody examination in the lungs of healthy people showed that there was a weak positive reaction with SMA antibody in the alveolar cells of the lung and it appeared as golden brown granules in the nucleus (Figure 3 (A) (B)). If, in the lungs of the group of patients with COVID-19, there was a strong positive reaction with SMA antibody in the cytoplasm of alveolar cells of the lung, which appeared as golden brown nuclear spots. The high and positive reaction of SMA antibody was shown as a new biomarker in the disease of COVID-19 and other pulmonary disorders. This positive reactivity can play a role in lung pathology and lung tissue destruction (Figure 3 (C) (D) (E) (F)).

The results of examining the Vimentin antibody in the

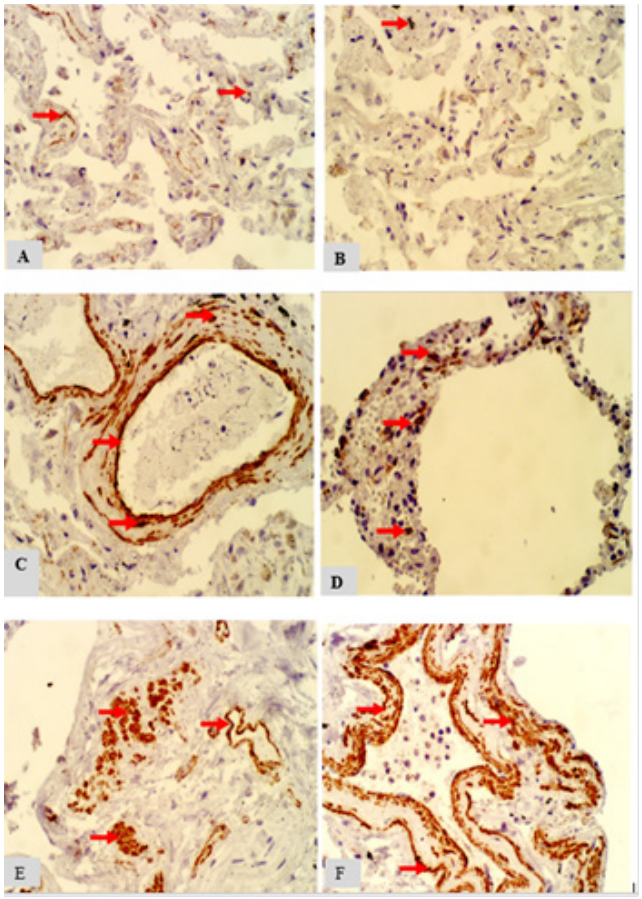

Figure 3. Normal lung group. (A) (B). Showed a weak positive reaction with SMA antibody in the alveolar cells of the lung which appear as golden-brown granules in the nucleus (arrow). IHC-SMA-DAB stain. 400x. **COVID-lung group. (C) (D) (E) (F).** Showed a strong positive reaction with *SMA* antibody in the nucleus of alveoli (**arrow**) which appear as golden-brown nuclear patches. IHC-*SMA*-DAB stain. 400x.

Table 2. The level of expression of antibodies in the covid-19 group and the group of healthy people.

Antibody	Covid- 19 group	Non-Covid- 19 group	P-value
BCL2 antibody	0.74 ±(0.25)	0.57 ± (0.34)	0.001
Catenin antibody	0.041	0.022	0.001
SMA antibody	0.12	0.03	0.001
Vimentin antibody	67kDa	57kDa	0.001

lungs of healthy people showed that there was no reaction in the lungs of these people (Figure 4 (A) (B)). While in the examination of the lungs of people with COVID-19, the results showed that there was a strong positive reaction with Vimentin antibody in the cytoplasm of lung alveolar cells, which appeared as golden brown cytoplasmic granules (Figure 4 (C) (D)). Vimentin is known as fibroblast intermediate filament, which showed a positive reactivity in COVID-19 patients, which can probably have a significant impact on the disease process.

The results of the analysis of gene expression showed that the mean BCL2 antibody expression in the group of Covid-19 patients was higher than in the group of healthy people, and this amount of antibody expression was significant ($P \leq 0.001$). The mean expression of Catenin antibodies in the group of Covid-19 patients was higher than the group of healthy people, and this amount of antibody expression is significant ($P \leq 0.001$). The mean expression of SMA and Vimentin antibodies in the group of Covid-19 patients was higher than that of healthy people, and this difference is also significant ($P \leq 0.001$) (Table 2).

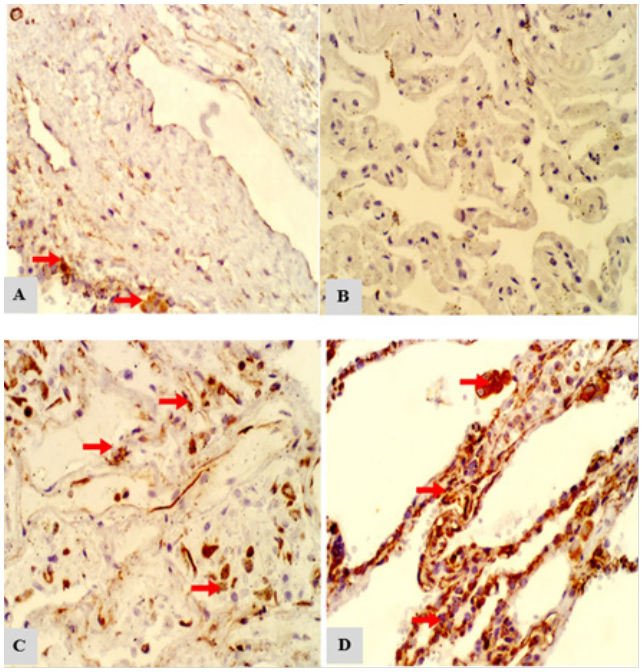

Figure 4. Lung, Normal lung group. (A) (B). Showed negative reaction with Vimentin antibody in the lung tissue. IHC-Vimentin-DAB stain. 400x. **Covid-lung group. (C) (D).** Showed a strong positive reaction with *Vimentin* antibody in the cytoplasm of alveolar cells of the lung (arrow) which appear as golden-brown cytoplasmic granules. IHC-*Vimentin*-DAB stain. 400x.

Discussion

This study was conducted to investigate and analyze the lung tissue of people who died due to the disease COVID-19 and compare it with healthy people.

Based on the results of immunopathology in deceased people, it was shown that there was a strong positive reaction with BCL2 antibody in the cytoplasm of lung alveolar cells compared to the lungs of healthy people. Also, a positive reaction with catenin antibody and SMA antibody occurred in the cytoplasm of alveolar lung cells in the lungs of the patients. And finally, it was shown that there was a Vimentin antibody reaction in the cytoplasm of the lung alveolar cells of patients.

Bcl-2 antibody is a protected anti-apoptotic protein that plays an important role in regulating cell death (apoptosis) by inhibiting (anti-apoptotic) or by inducing (pro-apoptotic) apoptosis [17,18](17,18).

Bcl-2 disorders are known as the cause of a number of cancers, including melanoma, breast, prostate, chronic lymphocytic leukemia, and lung, and it is also a possible cause of schizophrenia and autoimmunity (19,20). A study by L Lorente et al. (2021) (21) aimed to determine the relationship between the serum concentration of sFas and Bcl2 and the mortality of COVID-19 patients in Spain. Its results showed that patients with higher serum levels and positive reaction of Bcl-2 antibody have a higher mortality rate than healthy people and this result is consistent with the result of our study. In other studies, it was shown that changes in Bcl2 and increasing its level can be considered as a prognostic factor in the mortality of patients (22,23). In the study of (24) S André et al. (2022) also showed that Bcl-2 has changes in T cells of COVID-19 patients and is associated with a higher rate of mortality (24).

In the study by JR McGill et al (2022) (25), which was conducted with the aim of identifying antibody responses and potential antibody reactions in COVID-19 patients, It appears that anti-SARS-CoV-2 antibodies can cross-react with endogenous human proteins that drive some of the pathologies associated with COVID-19. In line with the results of our study, the results of this study showed that the anti-catenin antibody has high reactivity in COVID-19 patients and can play a significant role in the process of pathogenesis. Also, in the study of C Zhang et al. (2021) (26), it was shown that among the three pathways identified in lung tissues that were involved in lung fibrosis, one of the pathways was the WNT signaling pathway, which is associated with high reactivity of catenin antibody And these results were in line with the results of our study, which showed that the high reactivity of catenin antibody in lung tissue can play an essential role in lung tissue fibrosis.

The present study showed that there is a high reactivity in lung cells with SMA antibodies. In the study conducted by S Valdebenito et al. (2021) (27), there was a cellular immune response and SMA antibody reaction in the lungs of patients, which was the cause of inflammation, fibrosis of the lung tissue, and ultimately the high mortality rate in COVID-19 patients.

Vimentin antibody has been proposed as a possible cellular target for the treatment of COVID-19. The important role that vimentin plays in viral infection is well established: Vimentin has been reported as a receptor for the COVID-19 virus. Hemopenia is involved in virus replication in the cell and in both viral infection and the subsequent explosive immune inflammatory response, and lower vimentin expression is associated with the inhibition of epithelial-to-mesenchymal transition and fibrosis. The results of the study showed that vimentin antibody was associated with lung tissue fibrosis and increased mortality in COVID-19 patients compared to healthy individuals. In the study of Z Li et al. (2020) (28) it was shown that the rise and reactivity of vimentin antibodies were associated with the destruction of lung tissue, which is consistent with the results of our study.

The role of vimentin in maintaining cell integrity and resistance to various diseases is known. In the study of V Pandita et al. (2021) (29)**,** it has been shown that the Vimentin antibody plays an important role in the pathogenesis of COVID patients and can be considered as one of the therapeutic targets. In other studies, it was shown that vimentin can play an important and vital role in the disease process so that it affects the consequences of the disease (30,31).

The results of this study indicate an important spectrum of immunopathological disorders in COVID-19 patients. Each of the investigated factors showed well that they can play an important and key role in the disease process. Based on the results, it was shown that all four investigated factors BCL2, catenin, SMA antibody and vimentin antibody played an important role in inflammation and fibrosis of lung tissue in patients with Coivd and the association of these four factors with each other have played a significant role in aggravating the symptoms and worsening of the disease. And finally, the prognosis of these factors for COVID-19 patients can seem dangerous.

Acknowledgments
We would like to thank all the patients who patiently helped in this research.

Interest conflict
The authors have no conflicts of interest to declare.

Author's contributions
All authors passed the criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editors.

Data availability
The authors guarantee that the data of this research will be provided at the request of other researchers.

Financial disclosure
The authors declared that this study received no financial support.

References

1. Rendeiro AF, Ravichandran H, Bram Y, Chandar V, Kim J, Meydan C, et al. The spatial landscape of lung pathology during COVID-19 progression. Nature 2021;593:564–9.
2. Sterne JAC, Murthy S, Diaz J V, Slutsky AS, Villar J, Angus DC, et al. Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19: a meta-analysis. Jama 2020;324:1330–41.
3. Bogoch II, Watts A, Thomas-Bachli A, Huber C, Kraemer MUG, Khan K. Pneumonia of unknown aetiology in Wuhan, China: potential for international spread via commercial air travel. J Travel Med 2020;27:taaa008.
4. Maiese A, Manetti AC, La Russa R, Di Paolo M, Turillazzi E, Frati P, et al. Autopsy findings in COVID-19-related deaths: a literature review. Forensic Sci Med Pathol 2021;17:279–96.
5. Velavan TP, Meyer CG. The COVID-19 epidemic. Trop Med Int Heal 2020;25:278–80.
6. Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu Rev Virol 2016;3:237–61.
7. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033–4.
8. McElvaney OJ, McEvoy NL, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM, et al. Characterization of the inflammatory response to severe COVID-19 illness. Am J Respir Crit Care Med 2020;202:812–21.
9. Dhasmana D, Group R. Dexamethasone in hospitalized patients with Covid-19. N Engl J Med 2021.
10. Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, et al. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. Bmj 2020;369.
11. Sinha P, Calfee CS, Cherian S, Brealey D, Cutler S, King C, et al. Prevalence of phenotypes of acute respiratory distress syndrome in critically ill patients with COVID-19: a prospective observational study. Lancet Respir Med 2020;8:1209–18.
12. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol 2020;20:363–74.
13. Grasselli G, Tonetti T, Protti A, Langer T, Girardis M, Bellani G, et al. Pathophysiology of COVID-19-associated acute respiratory distress syndrome: a multicentre prospective observational study. Lancet Respir Med 2020;8:1201–8.
14. Da Silva SR, Ju E, Meng W, Paniz Mondolfi AE, Dacic S, Green A, et al. Broad SARS-CoV-2 cell tropism and immunopathology in lung tissues from fatal COVID-19. J Infect Di 2021.
15. Bösmüller H, Matter M, Fend F, Tzankov A. The pulmonary pathology of COVID-19. Virchows Arch 2021;478:137–50.
16. Zarrilli G, Angerilli V, Businello G, Sbaraglia M, Traverso G, Fortarezza F, et al. The immunopathological and histological landscape of COVID-19-mediated lung injury. Int J Mol Sci 2021;22:974.
17. Warren CFA, Wong-Brown MW, Bowden NA. BCL-2 family isoforms in apoptosis and cancer. Cell Death Dis 2019;10:177.
18. Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ 2018;25:65–80.
19. Dhyani P, Quispe C, Sharma E, Bahukhandi A, Sati P, Attri DC, et al. Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. Cancer Cell Int 2022;22:1–20.
20. Talebi M, Talebi M, Farkhondeh T, Samarghandian S. Biological and therapeutic activities of thymoquinone: Focus on the Nrf2 signaling pathway. Phyther Res 2021;35:1739–53.
21. Lorente L, Martín MM, González-Rivero AF, Pérez-Cejas A, Argueso M, Perez A, et al. Blood concentrations of proapoptotic sFas and antiapoptotic Bcl2 and COVID-19 patient mortality. Expert Rev Mol Diagn 2021;21:837–44.

22. Navratil V, Lionnard L, Longhi S, Combet C, Aouacheria A. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) envelope (E) protein harbors a conserved BH3-like motif. BioRxiv 2020:2004–20.
23. Yu-Jie DAI, Shi-Yao W, Shuai-Shuai G, Jin-Cheng L, Fang LI, Jun-Ping K. Recent advances of traditional Chinese medicine on the prevention and treatment of COVID-19. Chin J Nat Med 2020;18:881–9.
24. André S, Picard M, Cezar R, Roux-Dalvai F, Alleaume-Butaux A, Soundaramourty C, et al. T cell apoptosis characterizes severe Covid-19 disease. Cell Death Differ 2022;29:1486–99.
25. McGill JR, Lagassé HAD, Hernandez N, Hopkins L, Jankowski W, McCormick Q, et al. A structural homology approach to identify potential cross-reactive antibody responses following SARS-CoV-2 infection. Sci Rep 2022;12:11388.
26. Zhang C, Wu Z, Li J, Tan K, Yang W, Zhao H, et al. Discharge may not be the end of treatment: Pay attention to pulmonary fibrosis caused by severe COVID-19. J Med Virol 2021;93:1378–86.
27. Valdebenito S, Bessis S, Annane D, Lorin de la Grandmaison G, Cramer–Bordé E, Prideaux B, et al. COVID-19 lung pathogenesis in SARS-CoV-2 autopsy cases. Front Immunol 2021:3900.
28. Li Z, Paulin D, Lacolley P, Coletti D, Agbulut O. Vimentin as a target for the treatment of COVID-19. BMJ Open Respir Res 2020;7:e000623.
29. Pandita V, Ajila V, Babu GS, Hegde S, Asan MF. Vimentin and its role as a biomarker in health and disease. Gülhane Tip Derg 2021;63:232.
30. Malik N, Dhuldhaj UP (2023) Available drug therapies on COVID-19 and its side effects: An overview. Cell Mol Biomed Rep 3 (1): 41-58. doi: Https://doi.org/10.55705/cmbr.2022.364739.1071
31. Ghanem M, Homps-Legrand M, Garnier M, Morer L, Goletto T, Frija-Masson J, et al. Blood fibrocytes are associated with severity and prognosis in COVID-19 pneumonia. Am J Physiol Cell Mol Physiol 2021;321:L847–58.