Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Expression of antigen-presenting cells in lung of postmortem SARS-CoV-2 cases

Taban Kamal Rasheed*

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

Article Info

Article history:

Received: November 26, 2023
Accepted: April 05, 2024
Published: August 31, 2024

Use your device to scan and read the article online

Abstract

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is a deadly pulmonary disease with impaired immunological response that causes significant tissue damage and organ failure. Postmortem examination of the lung is a useful tool for understanding the immunopathogenesis of this virus. Lung autopsy samples from seven dead SARS-CoV-2 patients were obtained and evaluated using hematoxylin and eosin stain to analyze the histopathological changes in those samples, on the other hand, Immunohistochemical (IHC) staining was used for detection of CD21, CD1a, CR1 (CD35), CD68, Myeloperoxidase (MPO), CD15, CD56, CD3, CD20, CD4, and CD8 cells markers. Histopathological examination revealed diffuse alveolar damage with extensive parenchymal architecture distortion, intravascular fibrin clot, deposition of collagen fibers, vascular congestions and blood vessels containing thrombi, pneumocyte type II with inflammatory cell infiltration. The IHC staining for the innate immune cells such as antigen-presenting cells (APCs) including dendritic cells, Macrophages, and neutrophils showed a strong positive staining, while CD56 Natural killer (NK) cells showed negative staining. On the other hand, the specific immune cells including; CD20 B cells, CD3 T cells, and CD4 helper T cells, showed positive staining while CD8 Cytotoxic T cells showed negative staining. The lung autopsy samples from patients with COVID-19 confirmed the presence of APCs through the positive staining of CD21, CD1a, CD35, CD68, MPO, and CD15 expressed the virus recognition, proinflammatory cytokine production, and adaptive immune cells activation through CD3, CD4, and CD20 positive staining and the role of APCs in the severity of pulmonary infection and pathogenesis of SARS-CoV-2 infection however the absence of the CD56 NK and CD8 cytotoxic T explains the worse infection status for the patients.

Keywords: SARS-CoV-2, Lung autopsy samples, Antigen presenting cells (APCs), Histopathology, Immunohistochemical staining (IHC).

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the respiratory viruses that causes lung infection through inflammation and tissue damage that distort lung functions [1]. Asymptomatic or mild-to-moderate symptoms are the most common response to the infection while severe lung injury in the form of acute respiratory distress can occur following the cytokine storm. This is characterized by pulmonary involvement including reduced pulmonary diffusion, loss of lung compliance causing pulmonary edema, respiratory failure and death [2]. The activation of numerous immune cells result in the release of significant amounts of chemokine and cytokines that increase inflammation causing different organ damage [3]. Thus, the SARS-CoV-2 pandemic has made us aware of the importance of effective host immune response and the harmful consequences of immune dysregulation [4].

During viral infection, antigen-presenting cells (APCs) migrate to lung-draining lymph nodes, with the assistance of co-stimulatory molecules and the local cytokine environment, they activate the naive $CD4^+$ and $CD8^+$ T cells in the lymph node. Antigen-specific T cells multiply, differentiate, and migrate to the lung tissue where they either directly destroy infected cells or indirectly trigger the accumulation of additional immune cells [5, 6].

The most identified innate immune cells involved in pathophysiology of cytokine storm are neutrophils, macrophages, and dendritic cells (DCs). Pro-inflammatory cytokines has been found to be elevated in a group of hospitalized non-vaccinated SARS patients and it has been demonstrated that six cytokines of lung including IL-1β, IL-6, IL-17A, TGF-β, TNF-α, and IFN- γ, were implicated in the lung's inflammatory process [7].

A number of lung autopsy samples from patients who died after contracting SARS-CoV-2 infection revealed pulmonary epithelial and endothelial damage, dysfunction of the alveolar-capillary barrier and slowing in the lung tissue repair processes. It also diminished fibrinolysis causing widespread vascular thrombosis. Furthermore, excessive pulmonary fibrosis, loss of alveoli, and vascular remodeling were observed [8].

Diffuse alveolar damage (DAD) was the prominent pathological feature of severe COVID-19 cases. Histopathological findings from 32 autopsy COVID-19 samples showed both exudative and proliferative DAD in 75% of the patients, while 9% of them showed acute or organized

* Corresponding author.
E-mail address: taban.rasheed@su.edu.krd (T. K. Rasheed).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.8.6

DAD and the rest of the patients were exudative DAD although they had never been intubated [9].

Many researchers in our region tried to understand the pathology of SARS-CoV-2 infection through studying the systemic effect of the virus. Although the complication and severity of the disease start from lung tissues, few studies have been done on the lung tissue itself due to Ministry of Health policy restrictions with regards to the safety instructions to deal with the dead bodies that should disposed of immediately without further investigations. Therefore, more studies are required to understand the immunopathology of the virus, the lung immunological response, and the potential participation of APCs including DCs, macrophages, neutrophils and B cells in the pathogenesis and clinical outcomes in patients who died with COVID-19.

2. Material and Methods

2.1. Cases and Ethical Issues

Authorization and approval of the current study were granted by the College of Science Ethical Committee at Salahaddin University and the General Directorate of Health for the use of autopsy samples in Erbil, Iraq with approval No.45/431 on 28th of June 2022 and No. 24538 on 20th of September 2022 respectively.

Seven autopsy samples (three females and four males, aged 63.57±7.955 years) were taken from histopathology department of the Medico-Legal Institute in Erbil and Duhok. They were all confirmed positive for SARS-CoV-2 RNA infection by PCR at the time of hospital admission and died from respiratory failure. The twelve specimens were obtained in accordance with the medical committee guidelines of the hospital.

2.2. Lung tissue sampling, processing, and analysis

All lung lobes including the core and periphery were sampled. Tissue specimens were fixed in 10% formalin solution for 48 hours, processed, embedded in paraffin and stained with Hematoxylin and Eosin stain (Thermo Scientific, USA) according to the histopathology department protocols of the Medico-Legal Institute-Erbil [10]. In order to examine the histopathological alterations in each autopsy sample, the section was viewed under a microscope (Olympus BX40, Japan) and photographed.

The most representative areas in the lung specimens were also sent to PAR private hospital for Immunohistochemical (IHC) staining [11] with antibodies against CD3 (clone UCHT1, Fisher 14-0038-82) for identification of T-cells, CD20 (clone 2H7, Fisher 14-0209-82) for identification of B-cells, CD21 (clone 2G9, Fisher MA5-11417) for identification of B-cell and follicular dendritic cells (FDCs), and CD15 (clone MC-480 Fisher MA1-002) for identification of neutrophil cells, CD68 (clone KP1 Fisher MA5-13324) for identification of macrophage cells, CD1a (clone O10 Fisher MS-1858-R7) for identification of DCs, CR1 (CD35) (clone E11 Fisher MA5-13122) for identification of FDCs cells, MPO (clone MC-480 Fisher MA1-002) for identification of neutrophil and eosinophil. CD4 (clone 4B12, Fisher MA5-12259) for the identification of helper T-cells, CD8 (clone SP16, Fisher MA5-14548) for identification of cytotoxic T-cells, and CD56 (clone 56C04, Fisher MA5-11563) for identification of natural killer (NK) cell. Ready-to-use monoclonal antibodies (from Thermo Fisher Scientific, Tudor Road, Manor Park, Runcom, Cheshire WA7 1TA, UK) were used. The sections obtained were analyzed under magnified power (X100 and 400x) with high-resolution color microscope camera.

Two specialized pathologists did the histopathological evaluation and approval of each slide. The analysis of immunological markers and the features of immune cell responses was performed on the basis of staining intensity of the cell membrane. Staining intensity was assessed for mild (Weak positive), moderate (Positive), intense (Strong positive), or no expression (Negative).

3. Results

3.1. Clinical data

Postmortem lung autopsy samples were collected from the seven cases of confirmed SARS-CoV-2-positive patients who died between (2020-2021). All of the cases had respiratory failure, and multi-organ failure and all of them were on mechanical ventilator support. The clinical data and laboratory results for the patients are summarized in Table-1which showed elevated D-dimers, ferritin, Erythrocyte sedimentation rate (ESR), procalcitonin, and C-reactive protein.

3.2. Histopathological findings

The postmortem lung section from the cases showed distorted parenchymal architecture, proliferative hyperplasia of bronchial epithelia lining (pneumocytes type II), desquamation and sloughing of necrotic epithelial cells, intravascular fibrin clot, deposition of collagen fibers, with vascular congestions and blood vessels contains thrombi. The interstitium shows edema and fibrosis with chronic inflammatory cell infiltration. Multiple lymphocyte foci are seen in addition to parenchymal scarring most of the alveoli are dilated and many of them are damaged with diffuse alveolar damage (DAD) as shown in figures 1- 4.

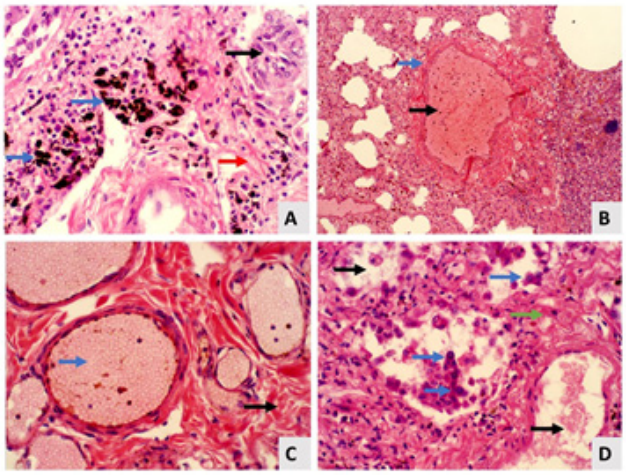

Fig. 1. Lung Autopsy Sample (H&E). (A) Showed multiple anthracosis lesions (blue arrow), collagen fibers deposition (red arrow), and hyperplasia of pneumocytes type II (black arrow) 400x. (B) Showed organized intravascular clot (black arrow) thickening of the vessel walls (blue arrow), focal interstitial infiltration of mononuclear inflammatory cells (red arrow) 100x. (C) Showed deposition of collagen fibers (black arrow), and organized vascular thrombi (blue arrow) 400x. (D) Showed serous exudate in alveolar space (black arrow), infiltration of inflammatory cells especially monocytes in alveolar space (blue arrow), interstitial infiltration of mononuclear inflammatory cells (green arrow) 400x.

Table 1. Clinical and demographic characteristics of the COVID-19 dead patients.

Parameters	Median (IQR) Percentage %	Minimum-Maximum
Age (Years) (Mean±S.D.)	63.00 (57.00-67.00) 63.57±7.955	55.00-79.00
Gender	Female 42.85% (3/7) Male 57.14%(4/7)	
BMI (kg/m^2)	27.39 (26.30-28.20)	25.73-28.41
Smoking	42.85% (3/7)	
Olfactory dysfunction (OD)	71.42% (5/7)	
Gustatory dysfunction (GD)	71.42% (5/7)	
Comorbidities		
Hypertension	71.42% (5/7)	
Cardiovascular disease	00.00% (0/7)	
Diabetes	28.57% (2/7)	
Medication taken		
Verapamil	71.42% (5/7)	
Verapamil +Insulin injection	28.57% (2/7)	
Required ICU Hospitalization	100.00% (7/7)	
C-reactive protein (CRP)(mg/l)	71.5 (67.69-85.60)	(57.60-88.79)
Ferritin (ng/ml)	850 (735-994.8)	(680-1035)
Procalcitonin (PCT) (ng/ml)	0.500 (0.400-0.800)	(0.300-1.800)
D-dimer (µg/ml)	7.700 (5.560-8.900)	(45.00-11.76)
E.S.R. (mm/hr.)	68.00 (56.00-75.00)	(45.00-76.00)
Lymphocyte (10^3/µl)	0.970 (0.837-1.050)	(0.783-1.200)
Neutrophil (10^3/µl)	14.83 (12.20-17.20)	(11.56-17.80)

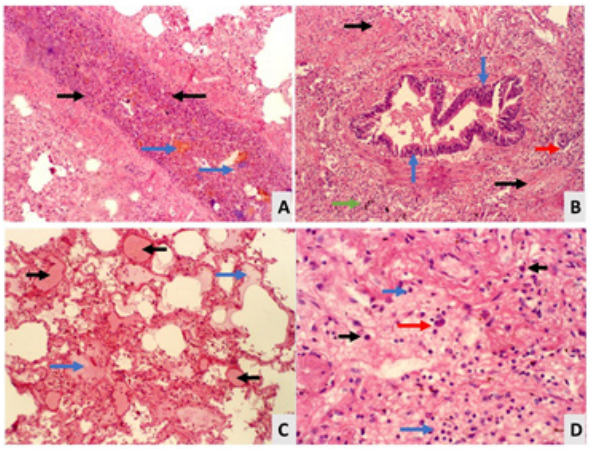

Fig. 2. Lung Autopsy Sample (H&E). (A) Showed Massive deposition of polymorphic inflammatory cells (black arrow) with interstitial hemorrhages (blue arrow) 100x. (B) Showed collagen fiber deposition (black arrow), hyperplasia of pneumocytes type II (blue arrow), anthracosis (green arrow), infiltration of myonuclear inflammatory cells (red arrow) 100x. (C) Showed multiple vascular clots (black arrow), serous exudate in alveolar lumen (blue arrow), diffuse alveolar damage (red arrow) 400x. (D) Showed infiltration of monocytes (black arrow), lymphocytes (blue arrow), and plasma cells (red arrow) 400x.

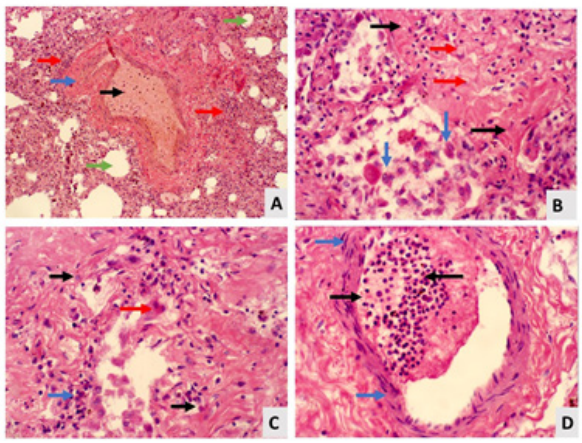

Fig. 3. Lung Autopsy Sample (H&E). (A) Showed intravascular fibrin clot (black arrow), thickening of vascular walls (blue arrow), Focal interstitial infiltration of mononuclear inflammatory cells (red arrow), Diffused alveolar damages (green arrow) 100x. (B) Showed deposition of fibrous tissue (black arrow), infiltration of plasma cells (blue arrow), interstitial edema (red arrow) 400x. (C) Showed infiltration of macrophages (black arrow), lymphocytes (blue arrow), and plasma cells (red arrow) 400x. (D) Showed numerous vascular intraluminal inflammatory cells (black arrow), and hyperplasia of fibrocytes (blue arrow) 400x.

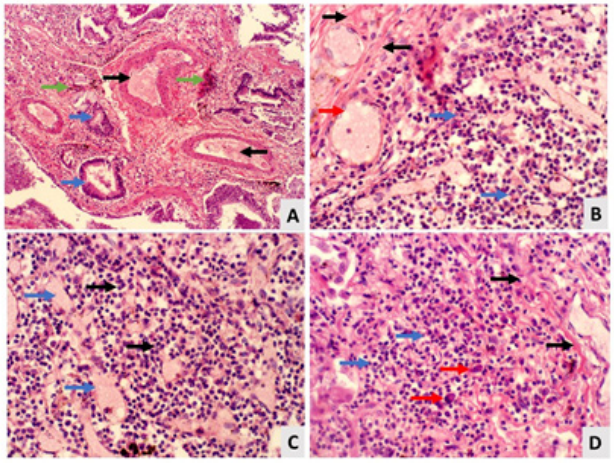

Fig. 4. Lung Autopsy Sample (H&E). (A) Showed Intravascular fibrin clot (black arrow), hyperplasia of pneumocytes type II (blue arrow), anthracosis (green arrow), infiltration of myonuclear inflammatory cells (red arrow) 100x. (B) Showed deposition of collagen fibers (black arrow), with infiltration of mononuclear inflammatory cells (blue arrow), organized thrombus (red arrow) 400x. (C) Showed interstitial infiltration of polymorphic nuclear cells (black arrow), and serous exudate (blue arrow) 400x. (D) Showed deposition of fibrous tissue (black arrow), infiltration of lymphocyte cells (blue arrow), and plasma cells (red arrow) 400x.

3.3. Immunopathological findings

The immunohistochemistry staining was done for CD21, CD1a, and CR1 (CD35), which are mainly expressed on the surface of dendritic cells. CD21 showed weak positive staining in most of the cases (A&B) and negative staining in some cases (C) while CD1a (D, E, F) and CD35 (G, H, I) showed strongly positive and positive staining in all of the cases (Fig.5).

Both CD68 (which is mainly expressed on the surface of macrophage) (A, B, C) and myeloperoxidase (MPO) (which is mainly expressed by neutrophils, eosinophil, and monocytes) (D, E, F) showed positive staining in all of the cases while CD15 which mediates neutrophil adhesion to dendritic cells (G, H, I) showed positive to weak positive staining (Fig.6).

CD3 T cell (which mainly expressed in both T-helper cells and T-cytotoxic cells) showed positive staining (A, B, C Fig.7) while CD20 (which mainly expressed on B-cells) showed strong positive staining ((D, E, F Fig.7). CD4 antibodies showed positive staining (Fig.7.G), while CD8 antibodies showed weak positive staining (Fig.7.H). IHC staining for CD56-ab showed negative staining (Fig.7.I).

4. Discussion

The lung is one of the organs that are directly in contact with the external environment. The APCs are considered to be an important regulator of immune response and stimulate antigen-specific $CD4^+$ and $CD8^+$ T-cells against infectious pathogens [12].

The immune response is triggered and activated when SARS-CoV-2 enters the cell through interaction with ACE2 receptor and completes its replication and proli-

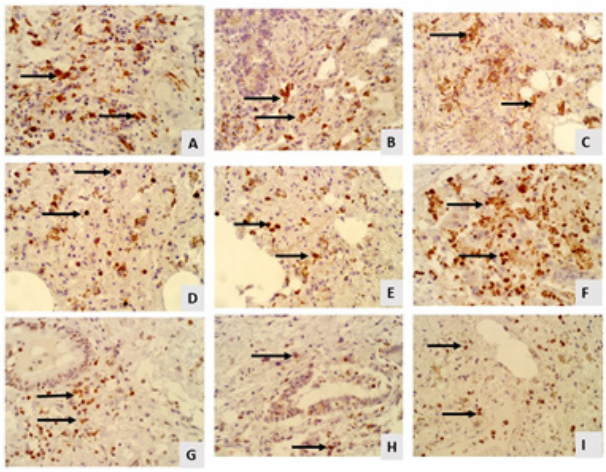

Fig. 6. Lung Autopsy Samples (IHC) showed strong positive staining for CD68 antibodies in some cases (A) and positive staining in most of the cases (B&C). MPO antibody staining showed positive staining in all the cases (D, E, F). CD15 antibodies showed positive staining in most cases (G&H) and weak staining in some cases (I). 400x.

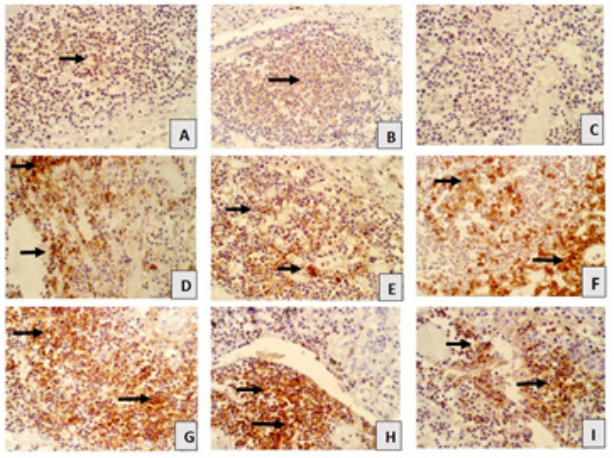

Fig. 5. Lung Autopsy Samples (IHC) staining with CD21 antibodies showed weak positive staining in most of the cases (A&B), while some cases (C) showed negative staining. CD1a antibodies showed strong positive in most cases (D&E) and positive staining in some cases (F). CD35 antibodies staining showed positive staining in all cases (G, H, I) 400x.

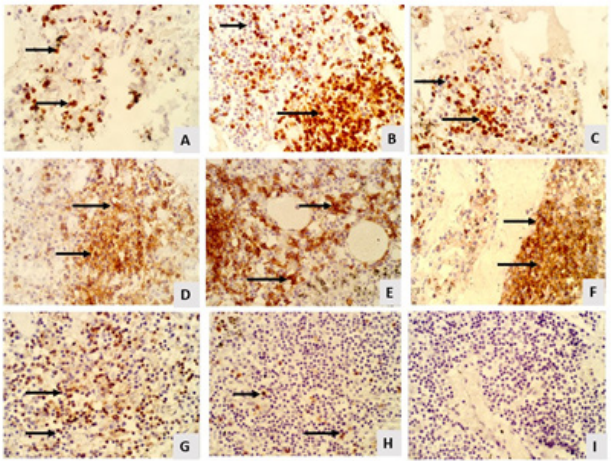

Fig. 7. Lung Autopsy Samples (IHC) showed positive staining for CD3 antibodies in all cases (A, B, and C). Staining for CD20 antibodies showed strong positive staining in all cases (D, E, F). IHC staining for CD4 antibodies showed positive staining (G), while CD8 antibodies showed weak positive staining (H). IHC staining for CD56 showed negative staining (I). 400x.

feration cycle. Phagocytic cells and different APCs such as dendritic cells (DCs), granulocytes, and macrophages serve to stimulate the innate immune system's activity [13].

DCs are divided into two categories: T-cell and B-cell-related DCs. The B-cell-related DCs are known as follicular dendritic cells (FDCs). While the T-cell-related DCs include interdigitating dendritic cells (IDCs), Langerhans cells, and connective tissue dendritic cells [14].

Histopathological observation indicated that the COVID-19 virus have a devastative cytopathic effects (Fig.1, 2, 3, and 4) which result from the immune responses including distorted parenchymal architecture, proliferative hyperplasia of bronchial epithelia lining (pneumocytes type II), desquamation and sloughing of necrotic epithelial cells, intravascular fibrin clot, deposition of collagen fibers, with vascular congestions and blood vessels contains thrombi. The interstitium shows edema and fibrosis with chronic inflammatory cell infiltration. All these findings were comparable to different studies done by different researcher [8, 15, 16].

Lung sections were immunostained for FDCs (through the complement receptor C3d -CD21) and the result showed weak positive in most of the cases, while some cases showed negative staining (Fig.5 A, B, and C). The complement receptor C3b (CD35) antibody staining showed a positive infiltration in the lung autopsy sample in all cases (figure 5- G, H, and I), while analysis done by other researchers [17] on DCs in lung tissues of COVID-19 patients showed an overall decrease in DCs subsets. On the other hand, IDCs that presented antigen to T-cell showed a strong positive infiltration in the lung autopsy sample as shown in Fig.5 D, E, and F. There is no comparable study could be found in regards to the DCs in SARS-CoV-2 infection, however a study was done by Elzbieta Radzikowska showed lung infiltration of IDCs in a young smoker suffering from Pulmonary Langerhans cell histiocytosis (PLCH) [18] and chronic smoker 76-year-old woman with lung adenocarcinoma [19].

Wendisch and his team concluded that SARS-CoV-2 causes significant acute fibroproliferative respiratory distress syndrome (ARDS) through macrophage responses [20]. The density of CD68-positive macrophage in the lung autopsy samples was high (Fig.6 A, B, and C). A similar result was observed by Jum'ah H and his colleague observed that there is a considerable increase in the density of CD68-positive macrophages [21].

One of the main cytokines in SARS-CoV-2 related to severe clinical problems is interleukin-6, which is mostly associated with macrophages and dendritic cells (DCs). This association not only highlights the importance of APCs but also the widespread involvement of the innate immune system in the development of the infection [22].

Myeloperoxidase (MPO), an enzyme primarily expressed in azurophilic granules, was immunostained in lung autopsy samples, and the results revealed a concentration of MPO-positive neutrophils (Fig.6 D, E, and F). The same result was reported by D'Agnillo and his team [8]. Lung injury is immediately caused by neutrophil toxicity and their degranulation through the release of leukotrienes and reactive oxygen species (ROS) as shown by the high quantities of neutrophils seen in the respiratory tract of COVID-19 patients. Through damaging the endothelial cells, which is one of the main targets of SARS-CoV-2, neutrophils, and can also contribute to systemic viral spread [23-26]. The density of CD15-myeloid and monocytic lineages of cells fluctuates from weak positive to strong positive (Fig.6 G, H, and I). This molecule engages in interactions with E-, L- and P-selectin enabling blood vessel attachment and subsequently movement away from the blood flow to the surrounding tissues [27].

Within the first few days of the viral infection, Human NK ($CD3^{-}CD56^{+}$) cells proliferated and increased in the lung [28]. The activated lung NK cells subsequently assist in the eradication of the virus by secretion of IFN-γ, the activation of adaptive immune cells (ADCC) and cytotoxic lysis, however many research indicated that production of IFN-γ at high-dose induces immunopathology in lungs infected with virus [29, 30]. NK cells in most of the lung autopsy samples showed negative infiltration in the lung section as shown in Fig (7-I). These cells are triggered during viral infections by infected cells (through contact-dependent processes) [31] and by cytokines such as TNF-α IL-15, IL-12, IL-2, and IL-6 [32, 33].

Additionally, we analyzed frequencies of specific immune cells in the lungs by immunostaining T cells (CD3+) of the CD4+ and CD8+ lineages. In line with previous observations [32], the density of CD3 showed enrichment in the lung tissue for CD3 T-cells (Fig.7 A, B, and C), while the specific B cells (CD20+) were selectively enriched in lung tissues of Covid-19 patients (Fig.7 D, E, and F). This result was comparable to a study done by Nienhold R. and his Colleague [34].

The T cells ($CD3^{+}$) of the $CD4^{+}$ lineages showed positive staining as shown in Fig. (7 G). Enriched infiltration of $CD4^{+}$ in the lung of the autopsy samples was reported by Valaebenito and his team [35] and Carsana and his colleagues [36].

T cells ($CD3^{+}$) of the $CD8^{+}$ lineages lymphocyte showed a weak positive (Fig.7 H) and this was comparable to study done by Yang Li and his colleague in 2020 which showed that lymphocytes particularly CD8+ T cells significantly decreased in severe cases compared with mild cases [37].

CD4 helper T cells are stimulated to secrete Th1 cytokine Interleukin-12 (IL-12) and IFN- interferon-γ (IFN-γ), this activation subsequently leads to $CD8^{+}$ cytotoxic T cell activation which may also activated through the direct binding with MHC class I on infected cells. The lack of a strong T cell response (especially, CD8 cytotoxic T cells) in the patients under analysis is crucial since it points to a possible systemic immunological malfunction. T-cell lymphopenia is seen in the blood of severe COVID-19 cases compared to those who are not infected [28, 38]. More samples undergoing studies to prove our point of view regarding CD56 and CD8.

5. Conclusions

In summary, following lung infection with SARS-CoV-2 virus, the antigen-presenting cells increase in number due to their migration from lung-draining lymph nodes, which triggered potent innate immune responses causing production of inflammatory cytokines such as TNF-α, IL-15, IL-6, IL-12, and IL-2. Secretion of IL-15 from the monocyte, macrophage, DCs, fibroblast, and all lung epithelial cells will stimulate the growth, proliferation and cytolytic activities of CD8 T cell and natural killer (NK) cells, therefore causing an increase in the expression of anti-apoptotic and decreasing the expression of pro-

apoptotic factors to prevent apoptosis [32]. This cytokine keeps the balance of the immune response and modulation of the disease condition.

When the concentrations of TNF-α and IL-15 increase within the normal level, this might decrease the danger of infection, however, when the concentration increases dramatically, the cytokine function will be reversed causing decrease in the proliferation and cytolytic activity of CD8 and NK cells with increase the apoptosis of these cells. This dramatic increase of these cytokines is probably the reason behind the worsening of infectious status of the infected patients.

Limitation

The main limitation is that we couldn't determine co-infection, as the bodies were not tested for any associated co-infection. The other limitation is the sample size as all COVID-19-related deaths, according to the Kurdistan MOH policy, were to be disposed of immediately without further investigations except these cases that we could obtain as they went under further forensic investigations as required by the Medico-Legal institution of Erbil and Duhok.

Funding

We did not support a specific source of funding for this article.

Declaration of competing interest

The authors declared that there is no conflict of interest related to this study.

Acknowledgments

The author thanks all the medical and administrative staff of Medico-Legal Institute- Hospital and special thanks to both histopathologist consultants who contributed to the evaluation & approval of findings.

References

1. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, W FS (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8(4):420-422. doi:10.1016/ S2213-2600(20)30076-X.
2. Gómez-Rial J, Rivero-Calle I, Salas A, Martinón-Torres F (2020) Role of monocytes/macrophages in Covid-19 pathogenesis: implications for therapy. Infec Drug Resist 13:2485-93. doi:10.2147. IDR.S258639.
3. Behrens EM, Koretzky GA(2017) Review:Cytokine Storm Syndrome: Looking Toward the Precision Medicine Era. Arthritis Rheumatol 69(6): 1135-1143. doi: 10.1002/art.40071.
4. Fajgenbaum DC, June CH (2020) Cytokine storm. N Engl J Med 383(23): 2255-2273. doi: 10.1056/NEJMra2026131.
5. Kim TS, Braciale TJ (2009) Respiratory dendritic cell subsets differ in their capacity to support the induction of virus-specific cytotoxic CD8+ T cell responses. PLoS One. 4(1): e4204. doi: 10.1371/journal.pone.0004204.
6. Kohlmeier JE, Cookenham T, Miller SC, Roberts AD, Christensen JP, Thomsen AR, Woodland DL (2009) CXCR3 directs antigen-specific effector CD4+ T cell migration to the lung during parainfluenza virus infection. J immunol 183(7):4378-84. doi:10.4049/jimmunol.0902022.
7. Romano A, Parrinello NL, Barchitta M, Manuele R, Puglisi F, Maugeri A, Barbato A, Triolo AM, Giallongo C, Tibullo D, Agodi LL, Palumbo GA, Raimondo FD (2022) In-vitro NET-osis induced by COVID-19 sera is associated to severe clinical course in not vaccinated patients and immune-dysregulation in breakthrough infection. Sci. Rep. 12(7237). doi:10.1038/s41598-022-11157-0.
8. D'Agnillo F, Walters KA, Xiao Y, Sheng ZM, Scherler K, Park J, Gygli S, Rosas LA, Sdtler K, Kalish H, Blatti CA, Zhu R, Gatzke L, Bushell C, Memoli Mj, O'Day SJ, Fischer TD, Hammond TC, Lee RC, Cash JC, Power ME, O'Keefe GE, Butnor KJ, Rapkiewicz AV, Travis WD, Layne SP, Kash JC, Taubenberger JK (2021) Lung epithelial and endothelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19. Sci Transl Med 13(620). doi: 10.1126/scitranslmed.abj7790.
9. Elsoukkary SS, Mostyka M, Dillard A, Berman DR, Ma LX, Chadburn A, Yantiss RK, Jessurun J, Seshan SV, Borczuk AC, Salvatore SP (2021) Autopsy findings in 32 patients with COVID-19: a single-institution experience. Pathobiol. 88(1): 56-68. doi: 10.1159/000511325.
10. Sheehan DC, Hrapchak BB (1987) Theory and Practice of Histotechnology. 2nd ed. Battelle Press. Columbus, Ohio.
11. Cuello AC (1993) Immunohistchemistry II. Wiley Press, NY.
12. Kawasaki T, Ikegawa M, Kawai T (2022) Antigen presentation in the lung. Front Immunol 13:860915 doi:10.3389/fimmu.2022.860915.
13. Zhao C, Zhao W (2020) NLRP3 inflammasome—a key player in antiviral responses. Front immunol 11:211. doi: 10.3389/fimmu.2020.00211.
14. Mesquita RA, Araújo VC, Paes RA, Nunes FD, de-Souza SC (2009) Immunohistochemical analysis for CD21, CD35, Caldesmon and S100 protein on dendritic cells types in oral lymphomas. J Appl. Oral Sci 3:248-53. doi:10.1590/s1678-77572009000300023.
15. Gheware A , Ray A, Rana D, Bajpal P, Nambirajan A, Mathur P, Trikha A, Arava S, Das P, Mridha AR, Singh G, Soneja M, Nischal N, Lalwani S, Wig N, Sarkar C, Jain D (2022) ACE2 protein expression in lung tissues of severe COVID-19 infection. Sci Rep 12: 4058 doi:10.1038/s41598-022-07918-6.
16. Nienhold R, Ciani Y, Koelzer VH, Tzankov A, Haslbauer JD, Menter T, Schwab N, Henkel M, Frank A, Zsikla V, Willi N, Kempf W, Hoyler T, Barbareschi M, Moch H, Tolnay M, Cathomas G, Demichelis F, Junt T, Mertz KD (2020) Two distinct immunopathological profiles in autopsy lungs of COVID-19. Nat Commun 11(1):5086. doi:10.1038/s41467-020-18854-2.
17. Sanchez-Cerrillo I, Landete P, Aldave B, Sanchez-Alonso S, Sanchez-Azofra A, Marcos-Jimenez A, Avalos E, Alcaraz-Serna A, de Los Santos I, Mateu-Albero T, Esparcia L, Lopez-Sanz C, Martinez-Fleta P, Gabrie L, Del Campo Guerola L, Calzada MJ, Gonzalez-Alvaro I, Alfranca A, Sanchez-Madrid F, Munoz-Calleja C, Soriano JB, Ancochea J, Martin-Gayo E. (2020) Differential redistribution of activated monocyte and dendritic cell subsets to the lung associates with severity of COVID-19. JCI. 130(12):6290-6300 doi:10.1172/JCI140335
18. Radzikowska E (2021) Update on pulmonary Langerhans cell histiocytosis. Front Med 7:582581. doi: 10.3389/fmed.2020.582581.
19. Khaliq MF, Noorani MM, Tariq SM, Koirala A, Mohamed HJC. (2020) Pulmonary Langerhans Cell Histiocytosis Associated with Bronchogenic Carcinoma. Cureus 12(1). doi: 10.7759/cureus.6634.
20. Wendisch D, Dietrich O, Mari T, von Stillfried S, Ibarra IL, Mittermaier M, Mache C, Chua RL, Knoll R, Timm S, Brumhard S, Krammer T, Zauber H, Hiller AL, Pascual-Reguant A, Mothes R, Bülow RD, Schulze J, Leipold AM, Djudjaj S, Erhard F, Geffers R, Pott F, Kazmierski J, Radke J, Pergantis P, Baßler K, Conrad C,

Aschenbrenner AC, Sawitzki B, Landthaler M, Wyler E, Horst D; Deutsche COVID-19 OMICS Initiative (DeCOI); Hippenstiel S, Hocke A, Heppner FL, Uhrig A, Garcia C, Machleidt F, Herold S, Elezkurtaj S, Thibeault C, Witzenrath M, Cochain C, Suttorp N, Drosten C, Goffinet C, Kurth F, Schultze JL, Radbruch H, Ochs M, Eils R, Müller-Redetzky H, Hauser AE, Luecken MD, Theis FJ, Conrad C, Wolff T, Boor P, Selbach M, Saliba AE, Sander LE (2021) SARS-CoV-2 infection triggers profibrotic macrophage responses and lung fibrosis. Cell. 184(26):6243-6261. doi: 10.1016/j.cell.2021.11.033.

21. Jum'ah H, Kundrapu S, Loeffler A (2022) The Density of CD-68 Positive Macrophages in the Epicardium Correlates with Covid-19 Infection in Deceased Patients. Mod Pathol.35(suppl 2): 7-8. ID: covidwho-1857323
22. McKechnie JL, Blish CA (2020) The innate immune system: fighting on the front lines or fanning the flames of COVID-19? Cell host microbe. 27(6): 863-869. doi: 10.1016/j.chom.2020.05.009.
23. Vardhana SA, Wolchok JD. (2020) The many faces of the anti-COVID immune response. J Exp Med. 217 (6). doi:10.1084/jem.20200678.
24. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, Daßler-Plenker J, Guerci P, Huynh C, Knight JS, Loda M, Looney MR, McAllister F, Rayes R, Renaud S, Rousseau S, Salvatore S, Schwartz RE, Spicer JD, Yost CC, Weber A, Zuo Y, Egeblad M. (2020) Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med 217(6). doi: 10.1084/jem.20200652.
25. Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan SJCr. (2020) Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol 39(7): 2085-2094. doi:10.1007/s10067-020-05190-5.
26. Hemmat N, Derakhshani A, Bannazadeh Baghi H, Silvestris N, Baradaran B, De Summa S (2020) Neutrophils, crucial, or harmful immune cells involved in coronavirus infection: a bioinformatics study. Front Genet 11: 641. doi: 10.3389/fgene.2020.00641.
27. Szlasa W, Wilk K, Knecht-Gurwin K, Gurwin A, Froń A, Sauer N, Krajewski W, Saczko J, Szydełko T, Kulbacka J, Małkiewicz B (2022) Prognostic and therapeutic role of CD15 and CD15s in cancer. Cancers. 14(9):2203. doi: 10.3390/cancers14092203.
28. Zhang Y, Wang X, Li X, Xi D, Mao R, Wu X, Cheng S, Sun X, Yi C, Ling Z, Ma L, Ning Q, Fang Y, Sun B, Wu D (2020) Potential contribution of increased soluble IL-2R to lymphopenia in COVID-19 patients. Cell. Mol. Immunol. 17(8): 878-80. doi: 10.1038/s41423-020-0484-x.
29. Xu X, Weiss ID, H Zhang HH, Singh SP, Wynn TA, Wilson MS, Farber JM (2014) Conventional NK cells can produce IL-22 and promote host defense in Klebsiella pneumoniae pneumonia. J Immunol. 192(4): 1778-86. doi: 10.4049/jimmunol.1300039
30. Cong J, Wei H. (2019) Natural killer cells in the lungs. Front immunol 10:1416. doi:10.3389/fimmu.2019.01416.
31. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W, Tian DS (2020) Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis 71(15): 762-768. DOI: 10.1093/cid/ciaa248.
32. Patidar M, Yadav N, Dalai SK (2016) Interleukin 15: A key cytokine for immunotherapy. Cytokine Growth Factor Rev 31:49-59. doi: 10.1016/j.cytogfr.2016.06.001.
33. Perpiñan C, Bertran L, Terra X, Aguilar C, Binetti J, Lopez-Dupla M, Rull A, Reverté L, Yeregui E, Gómez-Bertomeu F, Peraire J, Auguet T, On Behalf Of Covid-Study Group (2022) Resistin and IL-15 as Predictors of Invasive Mechanical Ventilation in COVID-19 Pneumonia Irrespective of the Presence of Obesity and Metabolic Syndrome. J. Pers. Med. 12(3): 391. https://doi.org/10.3390/jpm12030391
34. Nienhold R, Ciani Y, Koelzer VH, Tzankov A, Haslbauer JD, Menter T, Schwab N, Henkel M, Frank A, Zsikla V, Willi N, Kempf W, Hoyler T, Barbareschi M, Moch H, Tolnay M, Cathomas G, Demichelis F, Junt T, Mertz KD (2020) Two distinct immunopathological profiles in autopsy lungs of COVID-19. Nat Commun 11(1):5086. doi:10.1038/s41467-020-18854-2.
35. Valdebenito S, Bessis S, Annane D, Lorin de la Grandmaison G, Cramer-Bordé E, Prideaux B, Eugenin EA, Bomsel M (2021) COVID-19 lung pathogenesis in SARS-CoV-2 autopsy cases. Front. immunol. 12:735922. DOI: 10.3389/fimmu.2021.735922.
36. Carsana L, Sonzogni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P, Rech R, Colombo R, Antinori S, Corbellino M, Galli M, Catena E, Tosoni A, Gianatti A, Nebuloni M (2020) Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. Lancet Infect Dis (10): 1135-40. doi: 10.1016/S1473-3099(20)30434-5.
37. Yang L, Liu S, Liu J, Zhang Z, Wan X, Huang B, Chen Y, Zhang Y (2020) COVID-19: immunopathogenesis and Immunotherapeutics. Signal transduction and targeted therapy. Signal Transduct Target Ther 5(1):128. https://doi.org/10.1038/s41392-020-00243-2.
38. Cheng LL, Guan WJ, Duan CY, Zhang NF, Lei CL, Hu Y, Chen AL, Li SY, Zhuo C, Deng XL, Cheng FJ, Gao Y, Zhang JH, Xie JX, Peng H, Li YX, Wu XX, Liu W, Peng H, Wang J, Xiao GM, Chen PY, Wang CY, Yang ZF, Zhao JC, Zhong NS (2021) Effect of recombinant human granulocyte colony–stimulating factor for patients with coronavirus disease 2019 (COVID-19) and lymphopenia: A Randomized Clinical Trial. JAMA intern Med 181(1): 71-78. doi:10.1001/jamainternmed.2020.5503.