ADLİ TIP DERGİSİ
TURKISH JOURNAL OF FORENSIC MEDICINE

J For Med 2022;36(3):119-126
available online at: www.adlitipdergisi.com

Received: 19.09.2022
Accepted: 24.10.2022

ORIGINAL ARTICLE

FORENSIC PATHOLOGY

Postmortem histopathological findings in the spleen and the regional lymph nodes in SARS-CoV-2 positive cases

SARS-CoV-2 pozitif olgularda dalak ve bölgesel lenf düğümlerinde ölüm sonrası histopatolojik bulgular

Aytül Buğra[1], 0000-0001-5640-8329
Taner Daş[1], 0000-0002-1216-186X
Erdoğan Kara[2], 0000-0002-5695-5709
Nihan Ziyade[3], 0000-0002-3606-0756
Yalçın Büyük[4] 0000-0002-2270-5568

[1]Council of Forensic Medicine, Morgue Department, Histopathology Unit, Istanbul, Turkiye
[2]Council of Forensic Medicine, Morgue Department, Autopsy Unit, Istanbul, Turkiye
[3]Council of Forensic Medicine, Morgue Department, Microbiology Unit, Istanbul, Turkiye
[4]Council of Forensic Medicine, Istanbul, Turkiye

Corresponding author: Aytül Buğra, Council of Forensic Medicine, Morgue Department, Histopathology Unit, Istanbul, Turkiye.
E-mail : aytulsargan@gmail.com
Cite as: Bugra A, Das T, Kara E, Ziyade N, Buyuk Y. Postmortem histopathological findings in the spleen and the regional lymph nodes in SARS-CoV-2 positive cases. J For Med 2022;36(3):119-126.

ABSTRACT
Objective: *SARS-CoV-2 virus can affect the spleen and lymph nodes. In this study, we aimed to evaluate the histomorphological changes, immunohistochemical findings and real-time polymerase chain reaction test (rt-PCR) results in regional lymph nodes and spleen.*
Methods: *The regional lymph nodes and spleen samples of 12 cases of postmortem nasopharyngeal swabs that were positive for SARS-Cov-2 were evaluated. Upper paratracheal, lower paratracheal, and hilar lymph nodes and spleen samples were examined under a light microscope with H&E stained sections and immunohistochemically stained sections.*
Results: *Diffuse alveolar damage was seen in nine cases. Congestion, presence of immunoblastic and plasmablastic cells, expansion of subcapsular sinuses, presence of apoptotic cells, and sinus histiocytosis were the most common changes. The SARS-CoV-2 rt-PCR test was positive in four cases in the upper paratracheal lymph nodes, in seven cases in the lower paratracheal lymph nodes, and in five cases in the hilar lymph nodes. White pulp atrophy and red pulp hemorrhage were the most common findings in the spleen. The SARS-CoV-2 rt-PCR test was positive in four cases in the spleen.*
Conclusion: *SARS-CoV-2 virus can spread to the lymph nodes and spleen and destroy the tissues.*
Keywords: *SARS-CoV-2, spleen, lymph node, autopsy, forensic pathology*
ÖZET
Amaç: *SARS-CoV-2 virüsü dalak ve lenf düğümlerini etkileyebilir. Bu çalışmada bölgesel lenf nodları ve dalakta histomorfolojik değişiklikleri, immünohistokimyasal bulguları ve gerçek zamanlı polimeraz zincir reaksiyonu testi (rt-PCR) sonuçlarını değerlendirmeyi amaçladık.*
Yöntemler: *SARS-CoV-2 pozitif olan 12 postmortem nazofaringeal sürüntü vakasının bölgesel lenf düğümleri ve dalak örnekleri değerlendirildi. Üst paratrakeal, alt paratrakeal ve hiler lenf nodları ve dalak örnekleri ışık mikroskobu altında H&E boyalı kesitler ve immünohistokimyasal olarak boyanmış kesitler ile incelendi.*
Bulgular: *Dokuz olguda yaygın alveoler hasar görüldü. Konjesyon, immünoblastik ve plazmablastik hücrelerin varlığı, subkapsüler sinüslerin genişlemesi, apoptotik hücrelerin varlığı ve sinüs histiositozu en sık görülen değişikliklerdi. Dört olguda üst paratrakeal lenf düğümlerinde, yedi olguda alt paratrakeal lenf düğümlerinde ve beş olguda hiler lenf düğümlerinde SARS-CoV-2 rt-PCR testi pozitifti. Beyaz pulpa atrofisi ve kırmızı pulpa kanaması dalakta en sık görülen bulgulardı. Dalakta dört vakada SARS-CoV-2 rt-PCR testi pozitifti.*
Sonuç: *SARS-CoV-2 virüsü lenf düğümlerine ve dalağa yayılarak dokuları tahrip edebilir.*
Anahtar Kelimeler: *SARS-CoV-2, dalak, lenf nodu, otopsi, adli patoloji*

INTRODUCTION

Coronaviruses are single-stranded RNA viruses that cause a disease ranging from the common cold to severe acute respiratory syndrome. In 2019, a new coronavirus named SARS-CoV-2 was identified, and the disease caused by this virus was named as COVID-19 (1). As of 31 December 2021, more than 200 million people were infected and more than 5 million people died due to this disease (2).

The effects of COVID-19 on the lung have been shown in many studies (3,4). Studies in which changes in extrapulmonary organs are defined histopathologically are more limited. Lymph nodes and spleen are among the organs affected by this disease (5,6).

Lymphopenia is seen in 96% of severe COVID-19 patients and 80% in mild patients. It has also been shown that lymphopenia is associated with the severe course of the disease (7-10). The pathogenesis of lymphopenia in COVID-19 has been explained by many possible mechanisms. Possible mechanisms include extrapulmonary spread or direct invasion (11,12). It is important to reveal histopathological findings in order

to understand the changes in lymphoid organs and to explain the underlying mechanisms more clearly.

In this study, we aimed to evaluate the histomorphological changes, immunohistochemical findings and real time polimerase chain reaction test (rt-PCR) results in regional lymph nodes (upper paratracheal, lower paratracheal and hilar) and spleen samples of cases with postmortem SARS-CoV-2 PCR positivity.

MATERIALS AND METHODS

This study was carried out with the approval of the TR Ministry of Health General Directorate of Health Services Scientific Research Platform (2021-04-02T21_35_19) and the Education and Scientific Research Commission of the Council of Forensic Medicine (21589509/2021/334-30.103.2021).

The regional lymph nodes and spleen samples of 12 cases whose autopsies were performed at the Council of Forensic Medicine, Morgue Department between April 2021 and June 2021 and whose postmortem nasopharyngeal swabs were positive for SARS-CoV-2 were evaluated.

Histomorphological evaluation

Upper paratracheal, lower paratracheal and hilar lymph nodes and spleen samples were examined with a light microscope with H&E stained sections.

Immunohistochemical staining

BenchMark Ultra, Roche's fully automatic immunohistochemical slide staining system was used for the immunohistochemical procedures. CD68 (Dako FLEX Monoclonal Mouse Anti-Human CD68, Clone KP1, Ready to use), CD3 (Dako FLEX Polyclonal Anti-Human CD-3, Ready to use), CD4 (Dako FLEX Monoclonal Mouse Anti-Human CD4, Clone 4B12, Ready to use), CD20 (Dako FLEX Monoclonal Mouse Anti-Human CD20cy, Clone L26, Ready to use), CD8 (Dako FLEX Monoclonal Mouse Anti-Human CD8, Clone C8/144B, Ready to use) were performed on the spleen and lymph node tissues. CD20, CD3, CD4, and CD8 were used to differentiate the origin of the lymphocytic cells, and to assess and compare of the amount of T lymphocytes. CD68 was used to assess the amount of the histiocytes and to detect the hemophagocytic and lymphophagocytic histiocytes.

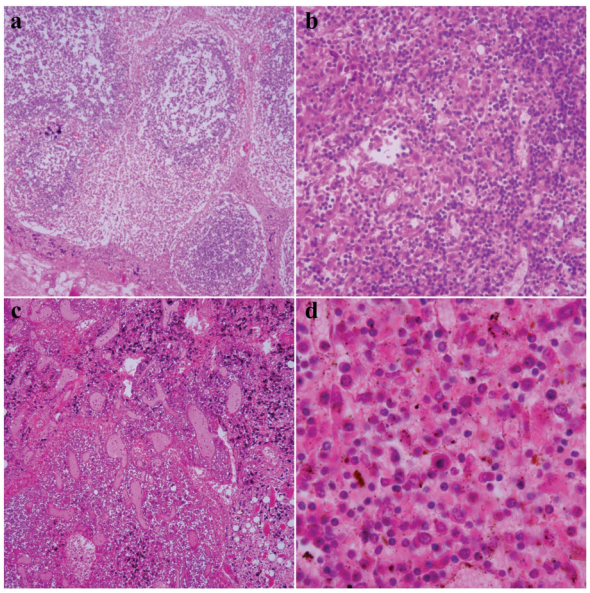

Figure 1: a. Germinal center reaction and sinus histiocytosis (H&E, x100), **b.** Sinus histiocytosis (HxE, x200), **c.** Congestion and sinus expansion (H&E, x100), **d.** Apoptotic lymphocyte (H&E, x1000)

SARS-CoV-2 RNA Real-Time Polymerase Chain Reaction

Postmortem Sars-CoV-2 RT-PCR assay, which is a nucleic acid amplification method that detects viral RNA, for COVID-19 were studied in nasopharyngeal swab, deep tracheal swab, lung swabs, and paraffin blocks of tissues. Nucleic acids were extracted on the QIASymphony (Qiagen / Germany) device using the "QIAsymphony DSP Virus / Pathogen midi" kit. "RealStar® SARS-CoV-2 RT-PCR Kit RUO (Altona Diagnostics, Hamburg, Germany)" was used by RT-PCR method and amplified in Rotor Gene (Qiagen / Germany) device in accordance with the manufacturer's recommendations.

Nucleic acids were extracted from paraffin-embedded formalin fixed lymph nodes and spleen tissues. Three or four 10 mm thick sections of each block were cut by a microtome. The xylene and ethyl alcohol deparaffinization procedure was applied to paraffin-embedded tissues. It was then incubated for 24 hours by adding 20 ml of proteinase K and 600 ml of ATL buffer solution.

Statistical analysis

SPSS (Statistical Package for the Social Sciences) V21 2012 program was used for statistical analysis. While evaluating the study data, descriptive statistical methods (Average, Standard Deviation, Median, Frequency, Ratio, Minimum and Maximum) were used.

RESULTS

Demographic characteristics of the autopsy cases

A total of 12 autopsy cases, eight male (66,7%) and four female (33,3%), were included in this study. The age of the deceased's was between 40 and 90 years (mean: 65,16 years). All of the cases had SARS-CoV-2 positivity in the postmortem nasopharyngeal swab sample. The baseline characteristics of the cases are given in Table 1 in detail.

Pathological changes in the lungs

Diffuse alveolar damage (DAD) was seen in nine cases. Three of these cases were in the exudative phase and six of them were in the proliferative phase of DAD. In two cases, there was no feature except edema. One case was not evaluated due to autolysis. In the microscopic examination of the lung, diffuse alveolar

Table 1: Baseline characteristics of the cases

Case	Age	Sex	Lung	rt-PCR results in the regional lymph nodes	rt-PCR results in the spleen
1	90	Male	Bronchopnemonia, Exudative phase of DAD	Upper paratracheal: Negative Lower paratracheal: Positive Hilar: Positive	Positive
2	56	Male	Lobular pneumonia, Proliferative phase of DAD	Upper paratracheal: Negative Lower paratracheal: Negative Hilar: Negative	Negative
3	67	Male	Bronchopnemonia, Exudative phase of DAD	Upper paratracheal: Positive Lower paratracheal: Positive Hilar: Positive	Negative
4	56	Male	Interstitial pneumonia, Proliferative phase of DAD	Upper paratracheal: Negative Lower paratracheal: Negative Hilar: Negative	Negative
5	76	Female	Lobular pneumonia, Proliferative phase of DAD	Upper paratracheal: Negative Lower paratracheal: Positive Hilar: Negative	Negative
6	84	Male	Interstitial pneumonia, Proliferative phase of DAD	Upper paratracheal: Negative Lower paratracheal: Negative Hilar: Negative	Negative
7	60	Male	Oedema	Upper paratracheal: Negative Lower paratracheal: Negative Hilar: Negative	Negative
8	78	Male	Oedema	Upper paratracheal: Negative Lower paratracheal: Negative Hilar: Negative	Negative
9	45	Male	Bronchopnemonia, Proliferative phase of DAD	Upper paratracheal: Negative Lower paratracheal: Positive Hilar: Negative	Negative
10	65	Female	Oedema, Exudative phase of DAD	Upper paratracheal: Positive Lower paratracheal: Positive Hilar: Positive	Positive
11	66	Female	Lobular pneumonia, Proliferative phase of DAD	Upper paratracheal: Positive Lower paratracheal: Positive Hilar: Positive	Positive
12	40	Male	Autolysis	Upper paratracheal: Positive Lower paratracheal: Positive Hilar: Positive	Positive

*DAD: Diffuse alveolar damage

damage was accompanied by lobular pneumonia (25%, n=3), bronchopneumonia (25%, n=3), and interstitial pneumonia (16.7%, n=2). The histopathological changes observed in the lungs are given in Table 1 in detail.

Pathological changes in the regional lymph nodes with immunohistochemical and rt- PCR findings

Histopathological changes in the upper paratracheal, lower paratracheal, and hilar lymph nodes, which are regional lymph nodes, were evaluated.

Congestion, presence of immunoblastic and plasmablastic cells, expansion of subcapsular sinuses, presence of apoptotic cells, and sinus histiocytosis were the most common changes seen in the upper paratracheal lymph nodes (Figure 1). Transformed cells were also observed in the upper paratracheal lymph nodes in five cases, hemophagocytosis in two cases, necrosis in one case, and subcapsular hemorrhage in

Table 2: Histopathologic findings of the regional lymph nodes

Histopathology	Upper paratracheal nodes (n/N)	Lower paratracheal nodes (n/N)	Hilar nodes (n/N)
Germinal center	3/12	5/12	5/12
Preserved architecture	3/12	4/12	4/12
Sinus histiocytosis	6/12	9/12	9/12
Granuloma	0/12	1/12	1/12
Antrachosis	4/12	9/12	10/12
Subcapsular sinus expansion	7/12	11/12	11/12
Necrosis	1/12	0/12	1/12
Subcapsular hemorrhage	1/12	2/12	0/12
Congestion	12/12	12/12	12/12
Immunoblast/Plasmablast	9/12	11/12	11/12
Transformed cells	5/12	7/12	7/12
Hemaphagocytosis/ Lymphophagocytosis	2/12	5/12	5/12
Apoptotic cells	6/12	10/12	10/12

Table 3: Histopathologic findings of the spleen

Histopathology	**n/N**
White pulp atrophy	8/12
Red pulp hemorrhage	7/12
Septic splenitis	2/12
Infarction	1/12
No specific finding	4/12

one case.

The most common changes in the lower paratracheal lymph nodes are congestion, presence of immunoblastic and plasmablastic cells, enlargement of subcapsular sinuses, presence of apoptotic cells, sinus histiocytosis, and anthracosis. Transformed cells in 7 cases, hemophagocytosis in 5 cases, subcapsular hemorrhage in 2 cases, and granuloma in one case were also seen in the lower paratracheal lymph nodes.

The most common changes in hilar lymph nodes were congestion, presence of immunoblastic and plasmablastic cells, enlargement of subcapsular sinuses, presence of apoptotic cells, anthracosis, and sinus histiocytosis. Transformed cells in 7 cases, hemophagocytosis in 5 cases, necrosis in one case, and granuloma in one case were also seen in the hilar lymph nodes. The histopathological changes observed in the lymph nodes are given in Table 2 in detail.

CD3, CD20, CD4, CD8, CD68 immunohistochemical staining was performed on lymph nodes. Sinus histiocytes stained positive with CD68. Staining was observed in paracortical T lymphocytes with CD3. Intense staining was seen in subcapsular areas with CD20. Staining was observed in paracortical T lymphocytes with CD4 and CD8. The number of CD4-stained lymphocytes was higher in paracortical T lymphocytes than in CD8-stained lymphocytes (Figure 2).

The SARS-CoV-2 rt-PCR test was positive in four cases in the upper paratracheal lymph nodes, in seven cases in the lower paratracheal lymph nodes, and in five cases in the hilar lymph nodes.

Pathological changes in the spleen with immunohistochemical and rt- PCR findings

Histopathological changes in capsular and hilar regions of spleen samples were evaluated. Similar morphological changes were seen in both regions. White pulp atrophy and red pulp hemorrhage were the most common findings (Figure 3). Septic splenitis was seen in two cases. Infarct was observed in one case. No specific finding was observed in four cases. The histopathological changes observed in the spleen are given in Table 3 in detail.

T lymphocytes stained with CD3, CD4 and CD8 were decreased in white pulp, and B lymphocytes stained with CD20 were decreased in both white and red pulp.

The SARS-CoV-2 rt-PCR test was positive in four cases in the spleen.

DISCUSSION

In this study, we described the histopathological and immunohistochemical changes in the spleen and lymph nodes of 12 autopsy cases with positive postmortem SARS-CoV-2 nasopharyngeal swab samples. We also showed the SARS-CoV-2 positivity in the tissues of these cases.

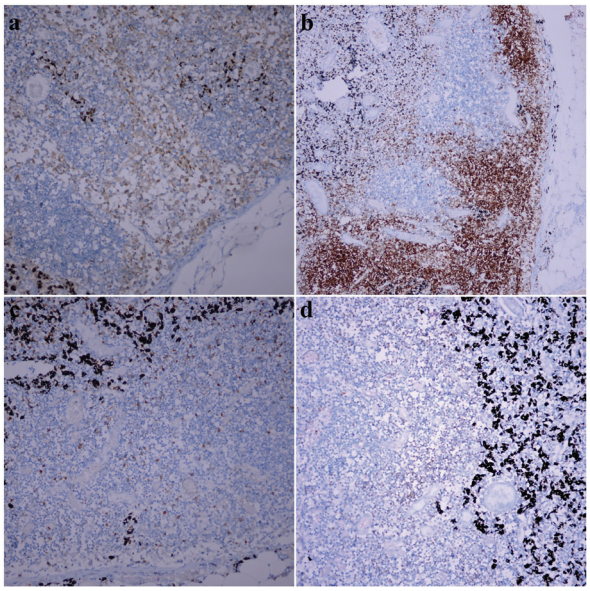

Figure 2: a. CD68 immunohistochemical staining of the lymph node (x200), **b.** Subcapsular CD20 immunohistochemical staining of the lymph node (x100), **c.** CD8 immunohistochemical staining of the lymph node (x200), **d.** CD4 immunohistochemical staining of the lymph node (x200)

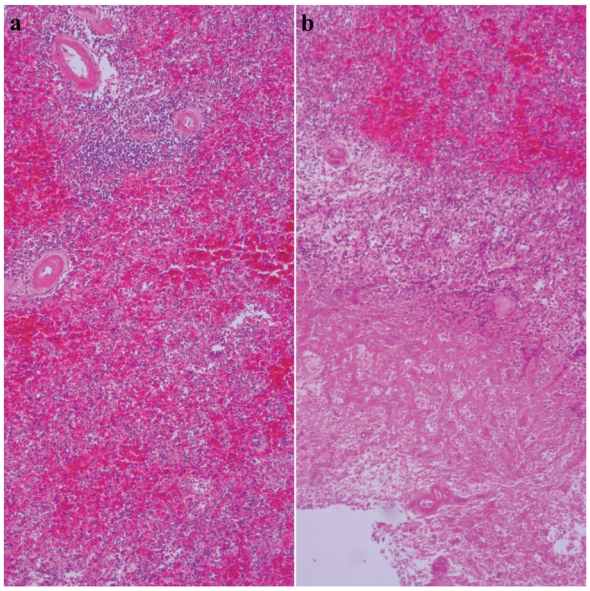

Figure 3: a. White pulp atrophy and red pulp hemorrhage (H&E, x200), **b.** Splenic infarction (H&E, x200)

As stated in many studies before, lung injury is the main cause of death in cases of COVID-19 (1,3,5,13). ACE-2 receptors, which are the main target of the SARS-CoV-2 virus, are found in many organs such as the lung, gastrointestinal system, heart, brain, vessels, spleen, kidney, and skin. This indicates that extrapulmonary organs can also be affected by the virus (6). Studies on the involvement of extrapulmonary organs are limited in the literature. While the number of autopsy studies on SARS-CoV-2 is gradually increasing in the literature, there are limited studies that examine the histopathology of the spleen and regional lymph nodes in detail.

The emergence of acute lung injury (ALI), systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS) in COVID-19 patients is a very important conditions because they can be mortal. Cytokines have been found to play an important role in the emergence of these clinical features (14-16). Inflammatory cytokines, which are released in excessive amounts from various tissues and cells by the cytokine storm, cause the immune system to lose its suppression of this situation, and these cytokines increase the arrival of other immune system cells to this region, resulting in increased organ damage (17). In severe cases of COVID-19, IL-6 and TNF-α are the key cytokines in the cytokine storm (18). IL-12, IL-1ß and IFN-γ are among the cytokines involved in the cytokine storm (17).

SARS-CoV-2, like other coronaviruses (MERS-CoV, SARS-Co-V), often causes lymphocytopenia. It has been shown that SARS-CoV causes lymphopenia by inducing inflammation and MERS-CoV infecting T cells (12). The mechanism of lymphocytopenia in the SARS-CoV-2 infection is not yet understood. The effect of SARS-CoV-2 virus on the hematopoietic system is being investigated through autopsy and clinical studies with a limited number of cases (11,12,19).

Histomorphological evaluation of the lymph node and spleen can help to understand both the effect of the virus on the tissues and the development of lymphopenia result from the extrapulmonary dissemination of the virus. In our study, we examined the changes in the regional lymph nodes by histomorphological evaluation and immunohistochemical staining. Dissemination of the SARS-CoV-2 virus to lymph nodes has been reported in previous studies (20). We also showed the presence of the virus in both regional lymph nodes and spleen in our study. DAD was present in all cases in which the virus was detected in the lymph nodes. We thought that this was the result of spread from the lung, which is the primary focus of inflammation. In three cases, the virus was detected in the regional lymph nodes, but not in the spleen. This may show us that the virus reaches the lymphoid tissues by lymphatic drainage from the lung. In one case, although there was autolysis in the lung, the presence of the virus in the lymph nodes and spleen were demonstrated. Since the evaluation could not be made due to autolysis in the lung, it was not possible to comment on whether the lymph node and spleen were directly infected or if there was extrapulmonary spread. In histomorphological evaluation, congestion and expansion of the sinuses were one of the common changes in lymph nodes. These findings were consistent with the literature (1,21). Studies have shown that

edema and plasmablast activation are prominent in early fatal cases, and germinal center reaction has been observed in cases with prolonged disease duration (19). Since the tissue samples of people who died due to COVID-19 were evaluated in our study, we thought that the frequent occurrence of plasmablast and immunoblastic cells is compatible with the literature. Likewise, germinal center reaction was less common.

Sinus histiocytosis and hemophagocytic lymphohistiocytosis (HLH) may develop after macrophage activation with a poor B cell response that bypasses the germinal center reaction due to impaired IFN response in COVID-19 (22). In our study, sinus histiocytosis, which is more common in the lower paratracheal and hilar lymph nodes, was among the remarkable findings. HLH is established with clinical laboratory and morphological findings. Pathological detection of hemophagocytosis is also a criterion in HLH. Infection-related HLH is most commonly caused by DNA viruses of the herpes virus family (such as EBV, CMV, HHV8). Although bone marrow is a typical diagnostic specimen, it can also be diagnosed from lymph nodes (23-25). In our study, we evaluated the lymph nodes because it is more practical and easy in terms of postmortem sampling. In a study, HLH associated with SARS-CoV-2 was defined in cases with ARDS and cytokine storm (26). In our study, the clinical and laboratory findings of the cases were not available. However, when the lungs of the cases with hemophagocytosis and lymphophagocytosis were examined, all of them had acute lung injury.

We demonstrated both sinus histiocytosis and hemophagocytosis by CD68 immunohistochemical staining. We thought that the hemophagocytosis observed in these cases is related to ARDS and cytokine storm.

TNF-α, one of the key cytokines of the cytokine storm, is in a negative correlation with the number of circulating lymphocytes in COVID-19 cases (20). The apoptosis effect of TNF-α has been explained by several mechanisms in studies. These mechanisms are specified as, TNF-α binding to TNFR-I, inducing apoptosis in T cells, TNF-α inducing T cell apoptosis in SARS-CoV-2 infection, TNF-α released from infected macrophages causing apoptosis in COVID-19 cases (26-28). In addition, the proapoptotic gene p53 level was found to be high in COVID-19 cases (29). Increased cytokine levels and increase in p53 level in cytokine storm may be among the factors causing lymphopenia by inducing apoptosis (30). Apoptosis was a finding in our study that was consistent with the literature. Although we do not know the lymphocyte count and cytokine levels in the blood, the histopathological findings of ARDS in the lung show us that the cases are in a cytokine storm. It supports that the apoptosis observed in these cases is associated with COVID-19.

When we reviewed the literature, we did not find any lymph node necrosis in studies on COVID-19 and lymph nodes. In our study, one case had both lymph node and spleen necrosis. However, in this case, extensive fibrin thrombus was present in the small vessels of the lung. In previous studies, it was stated that there is a predisposition to thrombosis in COVID-19 disease (5). We thought that this is a complication of the disease. With studies to be carried out in larger series, new results may emerge regarding whether necrosis is caused by a virus or a complication.

Another finding seen in lymph nodes in COVID-19 is loss of germinal centers. Lymphopenia and apoptotic effect caused by cytokine storm cause this situation (31). We also observed the loss of germinal center in our study. In the immunohistochemical staining, we observed CD20 (+) B lymphocytes in the subcapsular region of the lymph node. We observed staining of T lymphocytes in the parafollicular area with CD 3. When we look at the ratio of CD4 and CD8 staining, the ratio of staining with CD4 was higher.

In the literature, studies on the spleen as well as lymph nodes are limited. Studies have found findings that may be a direct effect of COVID-19, such as atrophy of the white pulp, histiocyte hyperplasia, and depletion in lymphoid follicles (32). In our study, similar findings were observed in the literature. Hyperplasia was detected by CD68 immunohistochemical staining. Lymphoid depletion and white pulp atrophy were demonstrated with CD3, CD20, CD4 and CD8. In addition, the microvascular thrombosis of the virus and the resulting necrosis have also been reported in studies (32). In one of our cases, there was necrosis as we mentioned before. Another finding was hemorrhage in the red pulp. Studies have also shown that hemorrhage may occur after the virus has damaged the spleen (32). We showed the presence of the virus in the tissues by rt-PCR. This also supports that the changes in the spleen are caused by viruses.

Our study has some limitations. Tissues were obtained at autopsy and were therefore accompanied by autolytic changes during evaluation. The blood lymphocyte levels, clinical features, histories, and drug use histories of the cases were unknown.

CONCLUSION

In our study, we showed that the virus can spread to the lymph nodes and spleen and cause damage to these tissues. We also supported the morphological changes we obtained with immunohistochemical findings. We think that autopsy studies are valuable in terms of providing new data on the mechanism of the disease along with clinical findings.

Funding: The authors received no financial support for the research and/or authorship of this article.

Conflicts of interest: Author AB, Author TD, and Author EK, Author NZ, Author YB declare that they have no conflict of interest.

Authors' contributions: All authors equally contributed to the study.

Scientific Commission approval: This study was carried out with the approval of the Council of Forensic Medicine Education and Scientific Research Commission. (21589509/2021/334-30.103.2021)

Acknowledgement: I thank the Council of Forensic Medicine for allowing us to do this work and for their support. Thank you to our staff in our Pathology Laboratory.

REFERENCES

1. Elsoukkary SS, Mostyka M, Dillard A, Berman DR, Ma LX, Chadburn A, et al. Autopsy Findings in 32 Patients with COVID-19: A Single-Institution Experience. Pathobiology. 2021; 88(1):56-68. doi:10.1159/000511325
2. WHO Coronavirus Disease Dashboard. https://covid19.who.int/. Accessed December 31, 2021.
3. Carsana L, Sonzogni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P, et al. Pulmonary post-mortem findings in a large series of COVID-19 cases from Northern Italy. Infect Dis 2020;20(10):1135-1140. doi:10.1101/2020.04.19.20054262.
4. Tian S, Hu W, Niu L, Liu H, Xu H, Xiao SY. Pulmonary pathology of early phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. J Thorac Oncol 2020;15:700-704. doi:10.1016/j.jtho.2020.02.010.
5. Satturwar S, Fowkes M, Farver C, Wilson AM, Eccher A, Girolami I, et al. Postmortem Findings Associated With SARS-CoV-2: Systematic Review and Meta-analysis. Am J Surg Pathol. 2021;45(5):587-603. doi: 10.1097/PAS.0000000000001650
6. Bugra A, Das T, Arslan MN, Ziyade N, Buyuk Y. Postmortem pathological changes in extrapulmonary organs in SARS-CoV-2 rt-PCR-positive cases: a single-center experience. Ir J Med Sci 2022;191(1):81-91. doi: 10.1007/s11845-021-02638-8
7. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N. Engl. J. Med 2020;382(18):1708-1720. doi:10.1056/NEJMoa2002032
8. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical Features of Patients Infected with 2019 Novel Coronavirus in Wuhan, China. Lancet 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
9. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al.Clinical Characteristics of 138 Hospitalized PatientsWith 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA 2020;323(11):1061-1069. doi:10.1001/jama.2020.1585
10. Huang I, Pranata R. Lymphopenia in Severe Coronavirus Disease-2019 (COVID-19): Systematic Review and Meta-Analysis. J. Intensive Care 2020;8:36. doi:10.1186/s40560-020-00453-4
11. Jafarzadeh A, Jafarzadeh S, Nozari P, Mokhtari P, Nemati M. Lymphopenia an Important Immunological Abnormality in Patients with COVID-19: Possible Mechanisms. Scand. J. Immunol 2021;93(2):e12967. doi:10.1111/sji.12967
12. Xiang Q, Feng Z, Diao B, Tu C, Qiao Q, Yang H, et al. SARS-CoV-2 Induces Lymphocytopenia by Promoting Inflammation and Decimates Secondary Lymphoid Organs. Front. Immunol 2021;12:661052. doi:10.3389/fimmu.2021.661052
13. Daş T, Buğra A, Arslan MN, Ziyade N, Buyuk Y. Evaluation of postmortem pathological changes in the lung in SARS-CoV-2 RT-PCR positive cases. J Surg Med 2021;5(11): 1113-1120. https://doi.org/10.28982/josam.997381
14. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China JAMA Intern Med 2020;180(7):934-943. doi:10.1001/jamainternmed.2020.0994
15. Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, Law KI, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study, Lancet 2003;361(9371):1767-1772. doi:10.1016/s0140-6736(03)13412-5
16. Nassar MS, Bakhrebah MA, Meo SA, Alsuabeyl MS, Zaher WA. Middle east respiratory syndrome coronavirus (MERS-CoV) infection: epidemiology, pathogenesis and clinical characteristics Eur Rev Med Pharmacol Sci 2018;22(15):4956-4961. doi:10.26355/eurrev_201808_15635 4956–4961
17. Song P, Li W, Xie J, Hou Y, You C. Cytokine storm induced by SARS-CoV-2. Clin Chim Acta, 2020;509:280-287. doi:10.1016/j.cca.2020.06.017
18. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol 2020;146(1):110-118. doi:10.1016/j.jaci.2020.04.006
19. Haslbauer JD, Matter MS, Stalder AK, Tzankov A. Histomorphological patterns of regional lymph nodes in COVID-19 lungs. Pathologe. 2021;42(Suppl 1):89-97. doi:10.1007/s00292-021-00945-6
20. Abdullaev A, Odilov A, Ershler M, Volkov A, Lipina T, Gasanova T, et al. Viral Load and Patterns of SARS-CoV-2 Dissemination to the Lungs, Mediastinal Lymph Nodes, and Spleen of Patients with COVID-19 Associated Lymphopenia. Viruses 2021;13(7):1410. doi:10.3390/v13071410
21. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. Histopathology 2020;77(2): 198–209. doi:10.1111/his.14134
22. Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. Nat Rev Immunol 2020;20(6):339–341. https://doi.org/10.1038/s41577-020-0321-6

23. Bergsten E, Horne A, Aricó M, Astigarraga I, Egeler RM, Filipovich AH, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: longterm results of the cooperative HLH-2004 study. Blood 2017;130(25):2728-2738. doi:10.1182/blood-2017-06-788349.

24. Risdall RJ, McKenna RW, Nesbit ME, Krivit W, Balfour HH Jr, Simmons RL, et al. Virus-associated hemophagocytic syndrome: a benign histiocytic proliferation distinct from malignant histiocytosis. Cancer 1979;44(3):993- 1002. doi:10.1002/1097-0142(197909)44:3<993::AIDCNCR2820440329>3.0.CO;2-5.

25. Ramos-Casals M, Brito-Zerón P, López-Guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. Lancet 2014;383:1503-1516. doi:10.1016/S0140-6736(13)61048-X.

26. Prilutskiy A, Kritselis M, Shevtsov A, Yambayev I, Vadlamudi C, Zhao Q, et al. SARS-CoV-2 Infection-Associated Hemophagocytic Lymphohistiocytosis. Am J Clin Pathol 2020;154(4):466-474. doi:10.1093/ajcp/aqaa124

27. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). Front immunol 2020;11:827. doi:10.3389/fimmu.2020.00827

28. Gupta S. Tumor necrosis factor-alpha-induced apoptosis in T cells from aged humans: a role of TNFR-I and downstream signaling molecules. Exp Gerontol 2002;37(2-3):293-299. doi:10.1016/s0531-5565(01)00195-4

29. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol 2017;39 (5):529-539. doi:10.1007/s00281-017-0629-x

30. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 2020;9(1):761-770. doi:10.1080/22221751.2020.1747363

31. Kaneko N, Kuo HH, Boucau J, Farmer JR, Allard-Chamard H, Mahajan VS, et al. Loss of Bcl-6-Expressing T Follicular Helper Cells and Germinal Centers in COVID-19. Cell 2020;183(1):143-157.e13. doi:10.1016/j.cell.2020.08.025

32. Feng Z, Diao B, Wang R, Wang G, Wang C, Tan Yet al. The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly decimates human spleens and lymph nodes. medRxiv. 2020. https://doi.org/10.1101/2020.03.27.20045427.