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CAMBIOS PATOLÓGICOS EN BIOPSIAS POSTMORTEM DE COVID-19

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RESUMEN

La epidemia de COVID-19 ha causado miles de muertes en todo el mundo. Se sabe que este virus produce una neumonía atípica que puede ser mortal. Existen pocos estudios sobre el examen patológico de tejidos de pacientes con COVID-19.

Objetivo: Describir los hallazgos patológicos en tejidos de pacientes fallecidos con infección por COVID-19.

Diseño: Se realizó un estudio de serie de casos con biopsias post mortem de pulmón, corazón e hígado de 18 pacientes con COVID-19 y se comparó los hallazgos con casos previamente reportados.

Resultados: Los principales hallazgos respaldan el cuadro histológico de fases exudativas y proliferativas coexistentes, de daño alveolar difuso (DAD), y la presencia de cambios de tipo citopático en los pulmones. También se encontró un aumento en el número de macrófagos alveolares en pacientes con valores elevados de ferritina, algo no descrito previamente. Diecisiete de los 18 casos mostraron depósitos de lipofuscina en el miocardio; la hipótesis es que esto podría estar relacionado con el tratamiento con hidroxiclороquina recibido en todos los pacientes. Ninguno de ellos presentó evidencia clínica documentada de cardiotoxicidad. Los hallazgos hepáticos se consideraron inespecíficos y se relacionaron con enfermedad preexistente, efecto terapéutico o falla sistémica.

Conclusiones: Los hallazgos del presente trabajo coincidieron con informes previos; además, se encontró un mayor número de macrófagos alveolares en pacientes con niveles más altos de ferritina y depósitos de lipofuscina en todos los corazones examinados, algo no reportado previamente.

PALABRAS CLAVE

COVID-19, SARS-CoV-2, neumonía, autopsia, daño alveolar difuso, injuria pulmonar aguda, síndrome de distrés respiratorio agudo del adulto

ABSTRACT

The COVID-19 epidemic has caused thousands of deaths all over the world. This virus is known to produce an atypical pneumonia that can lead to death. There are few studies concerning pathological examination of tissues from COVID-19 patients.

Objective: To describe the pathological findings in tissues from deceased patients with COVID-19 infection.

Design: A case series study was conducted, with postmortem biopsies of lung, hearth and liver from 18 patients with COVID-19 and compared findings with previously reported cases.

Results: The main findings support the histologic picture of coexisting exudative and proliferative phases of diffuse alveolar damage (DAD), and the presence of cytopathic-like changes in the lungs. An increased number of alveolar macrophages in patients with high ferritin values was also found, which has not been previously described. 17/18 cases showed deposits of lipofuscin in the myocardium; which, is hypothesized that could be related to treatment with hydroxychloroquine, received in all patients. None of them had documented clinical evidence of cardiotoxicity. Findings in the liver were considered not specific and related to preexisting disease, therapeutic effect and/or systemic failure.

Conclusions: The findings were congruent with previous reports; additionally, it was found higher number of alveolar macrophages in patients with highest levels of ferritin and deposits of lipofuscin in all the hearts examined, a finding not previously reported.

KEYWORDS

COVID-19, SARS-CoV-2, Pneumonia, Autopsy, Diffuse alveolar damage, Acute lung injury, Acute respiratory distress syndrome



CASE SERIES AND LITERATURE REVIEW

Pathological Changes in Postmortem Covid-19 Biopsies

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Abstract

The COVID-19 epidemic has caused thousands of deaths all over the world. This virus is known to produce an atypical pneumonia that can lead to death. There are few studies concerning pathological examination of tissues from COVID-19 patients.

Objective: To describe the pathological findings in tissues from deceased patients with COVID-19 infection.

Design: We conducted a case series study with postmortem biopsies of lung, heart and liver from 18 patients with COVID-19 and compared our findings with previously reported cases.

Results: Our main findings support the histologic picture of coexisting exudative and proliferative phases of diffuse alveolar damage (DAD), and the presence of cytopathic-like changes in the lungs. We also found an increased number of alveolar macrophages in patients with high ferritin values, which has not been previously described. 17/18 cases showed deposits of lipofuscin in the myocardium; which, we hypothesize that could be related to the treatment with hydroxychloroquine received in all patients. None of them had documented clinical evidence of cardiotoxicity. Findings in the liver were considered not specific and related to preexisting disease, therapeutic effect and/or systemic failure.

Conclusions: Our findings were congruent with previous reports; additionally, we found higher number of alveolar macrophages in patients with highest levels of ferritin and deposits of lipofuscin in all the hearts examined, most likely associated with hydroxychloroquine therapy.

Keywords

COVID-19, SARS-CoV-2, Pneumonia, Autopsy, Diffuse alveolar damage, Acute lung injury, Acute respiratory distress syndrome, Heart, Liver, Coronavirus

Introduction

The coronavirus-19 (COVID-19) pandemic has imposed a tremendous pressure on the medical community, who faces the challenge of treating critically ill patients while at the same time developing new knowledge about the pathogenesis, clinical presentation, treatment and prognostic factors of this disease. Since the beginning of the pandemic there has been a call to perform pathological studies for a better understanding of the disease [1] and recommendations for handling and processing the biological specimens [2].

From the first publication of pathological findings in COVID-19 tissues in March 23 [3] until June 2021, we gathered 36 papers including case reports, case series and letters to the editor from different electronic databases. There is urgency to introduce new knowledge among the scientific community to provide supportive evidence for clinical decisions; However, it has also been argued that safety precautions and overload of work in care units hamper the efforts to collect tissues for more pathological studies [4].



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We present a case series of postmortem biopsies from COVID-19 patients and a literature review of the pathological changes described in previous reports.

Methods

Cases

Eighteen deceased patients diagnosed with COVID-19 and treated at the Naval Medical Center, in Lima, Peru were included in the study. In all cases, a percutaneous biopsy without ultrasound guidance was performed within 2 hours post-mortem, between April 22nd and May 7th, 2020.

Our main goal was to obtain tissue from lungs, heart and liver; however, in obese patients the procedure was limited. Information corresponding to age, sex, duration of symptoms, test used for diagnosis, length of hospitalization, pulmonary CT scan, values of Ferritin and D-Dimer, and comorbidities was extracted from the medical records. In cases with more than one value of Ferritin and/or D-dimer, we chose the highest value (Table 1).

Ancillary studies

Tissues were studied with H & E stain and immunohistochemistry with CD3 (Bio SB, clone RBT), CD20 (Bio SB, clone L26), CD68 (Bio SB, clone KP-1), Cytokeratin 7 (Bio SB, clone OY-TL12/30), TTF-1 (Bio

SB, clone 8G7G3/1), and CD61 (Bio SB, Clone F2F). Immunohistochemistry was performed in 5 um sections embedded in paraffin, with antigen retrieval solution at 95 °C for 30', and using the BIO SB Mouse/Rabbit DAB HRP Brown Polydetector system[®].

Ethical considerations

The biopsies were collected during the first wave of COVID-19 and were aimed to obtain relevant information with clinical application in moments when little data was available. According to two documents published by the WHO, informed consent can be waived for research in the setting of a pandemic [5,6]. Based on these documents, the Ethical Committee of the Naval Medical Center approved the study.

Literature review

We collected all the papers describing the pathological findings in organ damage by COVID-19 published until June 2021. The sources were PubMed and Google Scholar. Some papers were not included in the review since they did not contain histopathological images and/or detailed pathological descriptions and others due to being published in Chinese language.

Results

Eighteen patients (17 males and 1 female) with a mean age of 65 years were included. The youngest

Table 1: Clinical and laboratory data.

N°	Age	Sex	DIAGNOSIS			Symptoms ¹ (days)	Hosp ² (days)	Ferritin ³ (ng/mL)	D-dimer ⁴ (mg/dL)	COMORBIDITY
			MT ⁵	RT ⁶	OTHER ⁷					
1	72	M	POS	NEG	X	8	6	1542	0.65	NO
2	56	M	POS	NEG	X	2	2	217	0	NO
3	50	M	POS	ND	X	11	12	4.49	2.67	RI
4	31	M	ND	POS IgM	X	2	2	ND	0.78	B-cell leukemia
5	72	M	POS	ND	X	7	2	942.4	> 4.6	DM
6	72	M	ND	POS IgG	X	5	5	1433	35.8	HT
7	55	M	POS	ND	X	5	18		3.36	HT, Obesity
8	78	M	ND	ND	X	5	5	870	0.79	NO
9	69	M	ND	ND	X	4	5	11500	1.52	NO
10	68	M	ND	POS IgG/IgM	X	10	4	1132.1	4.31	HT, Obesity
11	55	M	POS	NEG	X	NR	8	416	0.24	NO
12	65	M	ND	POS IgG/IgM	X	13	5	9326	1.3	Asma
13	78	M	ND	POS IgG/IgM	X	NR	9	705	1.25	DM
14	72	M	ND	NEG	X	8	19	10140	2.52	HT
15	51	F	POS	ND	X	6	16	818.2	0.8	NO
16	65	M	POS	NEG	X	10	16	1225	0.16	HT, Obesity, MI
17	91	M	POS	POS IgG/IgM	X	3	8	770	31.9	NO
18	63	M	ND	POS IgG/IgM	X	15	10	942	7.47	NO

¹Days of symptomatology, before admission; ²Days in hospital, before decease; ³Maximum value of Ferritin; ⁴Maximum value of D-Dimer; ⁵Molecular test; ⁶Rapid Test; ⁷Computed tomography and/or chest X-ray of atypical pneumonia; ND: Not Done; POS: Positive; NEG: Negative; NR: Not Referred; RI: Renal Insufficiency; DM: Diabetes Mellitus; HT: Hypertension; MI: Myocardial Infarction. Normal values of Ferritin: 12 - 300 ng/mL (men); 12 - 150 ng/mL (women). Normal values of D-dimer: < 0.5 mg/dL. Rapid Test: COVID-19 IgM/IgG Combo SD Biocensor[®]. Molecular test.

was a 31-year-old man with a previous diagnosis of B-cell leukemia and the oldest was a 91-year-old. Nine patients had a positive molecular test, five were positive for IgM and IgG with the rapid test, one was only IgG positive and another was only IgM positive. In three patients the diagnosis was established on the basis of a computed tomography and/or chest X-ray showing atypical pneumonia, after excluding differential diagnoses (Table 1).

All the patients had diffuse alveolar damage (DAD). The microscopic findings associated to DAD were divided by exudative and proliferative phase. 17 patients presented with hyaline membranes and 15 had neutrophilic infiltrates inside alveoli, consistent with the exudative phase of DAD (Table 2).

16 cases presented fibroblastic proliferation with dilated interstitial capillaries in the proliferative phase. Squamous metaplasia was found in 7 patients and epithelial hyperplasia in 5 (Table 2).

The most common finding in the heart was perinuclear lipofuscin granules inside myocytes. The content of these granules was confirmed with a positive PAS (Periodic Acid-Schiff) and negative Perl's staining (Figure 1).

On liver tissue, our patients had dilated sinusoids and erythrocyte congestion. Hepatic steatosis was found in all cases, except one.

Literature Review

36 papers, including as case reports, case series and

Table 2: Pathological changes

PATHOLOGICAL CHANGES		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	TOTAL			
LUNGS	EXUDATIVE PHASE	ALVEOLI	Dilated	x	x	x	x	x	x	x	x	x	x	x	x	x					15		
			Oedema							x								x					2
			Hyaline membranes	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		17
			Neutrophils	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x			15
		INTERSTITIUM	Expansion and distortion	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		18
			Capillary congestion																x		x		2
			Oedema			x																	1
			Thrombi	x	x		x	x	x	x	x	x	x	x	x	x				x		x	14
			Lymphocytic infiltration												x				x				3
			Dilated and rigid														x	x		x	x	x	5
	PROLIFERATIVE PHASE	ALVEOLI	Epithelial hyperplasia												x	x	x		x		x	5	
			Squamous metaplasia				x			x					x	x			x		x	x	7
			Dilated capillary	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	18
		INTERSTITIUM	Hyalinization of vessel walls													x	x			x		x	4
			Fibroblastic proliferation	x	x	x		x	x	x	x	x	x	x	x	x	x			x	x	x	16
			Hyaline globules																x				2
			Cytopathic-like changes	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	18
			Macrophages	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	18
			Multinucleated giant cells	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	18
			Red cell extravasation											x									2
OTHER	Intraalveolar abscesses					x	x	x	x	x										x	6		
	Interstitial abscesses																		x	x	3		
HEART	Lipofuscin	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	18		
	Inflammatory infiltrates	x	x						x	x	x	x	x			x	x	x	x		11		
	Microabscesses	x																			1		
LIVER	PARENCHYMA	Steatosis	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	17		
		Dilated	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	18	
	SINUSOIDS	Red cell congestion	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	16	
		Neutrophils																		x		1	
	PORTAL TRIAD	Thrombi					x					x										2	
		Red cell congestion					x															1	
		Periportal lymphocytic infiltrates										x			x		x	x				4	

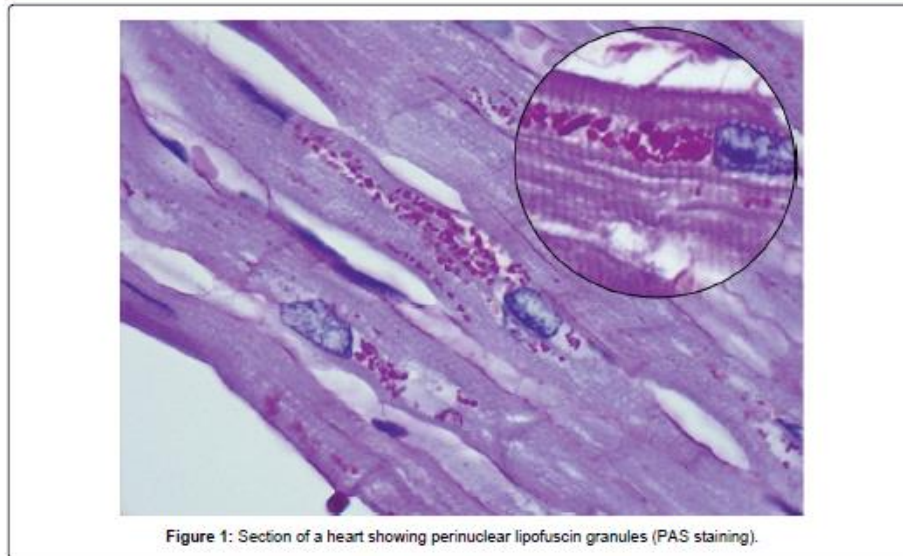


Figure 1: Section of a heart showing perinuclear lipofuscin granules (PAS staining).

letters to the editor, described pathological findings from autopsies, postmortem and *in vivo* biopsies in different organs. They revised in total 615 cases (367 males, 171 females, and 77 cases with no genre specified). 36 studies included lung biopsies and the others described findings in the spleen, kidney and heart.

The most common pathology in the lung was DAD, which was reported in 26 studies. Hyaline membranes and pneumocyte hyperplasia were associated to DAD in many cases [7-11]. Interstitial inflammation with predominant T lymphocytes and bronchial squamous metaplasia were also described [8,12].

Viral particles inside pneumocytes and renal cells were found in electron microscopy, however, only Yao, et al. and Borczuk, et al. confirmed SARS-CoV-2 origin of the particles using IHC [13,14].

Several studies reported thrombotic events in lungs, heart, kidney and spleen. In the lung, 14 studies found microthrombi in alveolar capillaries and arterioles. Other findings were intra-alveolar fibrin and fibrinous exudates.

Only two studies performed biopsies in living patients. Tian, et al. reported incidental findings in two patients with pulmonary resections for lung cancer who, inadvertently, contracted COVID-19 infection just before lung surgery [15]. The other study was performed on kidney biopsies by Ferlicot, et al., who described collapsing glomerulopathy and immunoglobulin deposits, acute tubular injury (ATI) and interstitial lymphocytic inflammation [16-18].

The renal findings described by Ferlicot, et al. were

similar with the reported in postmortem studies. The most frequent pathologies in the kidneys were ATI with tubular cell atrophy and collapsing glomerulopathy associated with loss of podocyte foot processes [17-20].

Table 3 summarizes the information from the papers included in this review.

Discussion

In this study, we described the microscopic features found in autopsies from 18 patients who died from SARS-CoV-2 infection at the beginning of the pandemic in the Naval Medical Center located in Lima, Peru. All but one of our cases were males, which is an unusual finding in a third-level hospital that covers a population of both men and women at all ages.

In 9 of our cases, the diagnosis was confirmed with a positive PCR for SARS-CoV-2 and in 7 patients, serologic testing was used. In 3 patients, the diagnosis was made based on the clinical symptoms and a CT scan. These patients could not access to a molecular or rapid testing due to the scarcity of specific testing for Covid-19 at the time of diagnosis. In the setting of a world pandemic, the positive predictive value of a CT scan for diagnosing Covid-19 infection is 69% and the negative predictive value is 89% [21]. For this reason, the 3 cases were included in our series.

We performed a literature review and contrasted our findings with the current literature. The majority of studies describing pathological changes associated with COVID-19 infection have focused on the lungs. To the best of our knowledge, there are 36 postmortem studies and only 1 study [15] describing the pathological

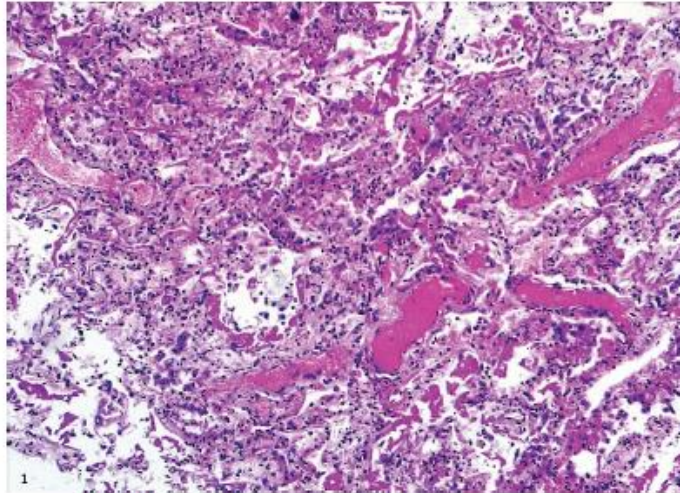


Figure 2: Prominent DAD with coexistence of exudative and proliferative phases.

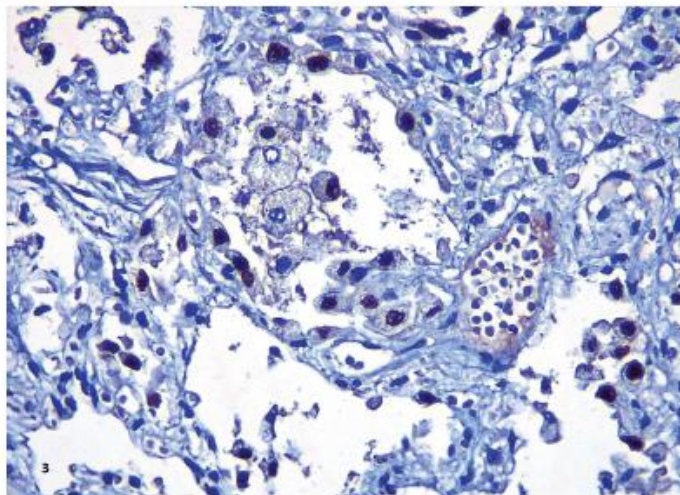


Figure 3: The immunohistochemistry with TTF-1 highlighted the hyperplasia of atypical type II pneumocytes.

findings in lung tissue from alive patients. The study on fresh tissue presented changes reported in the majority of postmortem studies and not related to death. These changes included: diffuse alveolar damage [10,12,14,22-26] alveolar proteinaceous exudates, vascular congestion [11,12,27] lymphocytic inflammatory infiltrates [8,11,13,23,24,28] hyaline membranes [7,9,12,20,24,26,28-33] and intraalveolar fibrin with

mononuclear and giant multinucleated cells [7], pneumocyte hyperplasia [7-11,14,15,24,25,28,33-35] alternating zones of exudative and proliferative phases of DAD, and suspected viral inclusions [7,20,22,36,37] (Table 3). Other pathological changes were thrombosis of small lung arteries [13,15], squamous metaplasia [12,24], bronchopneumonia [12,20], spherical secretions or globules [7], and megakaryocytes

[22,31]. Using immunohistochemistry and electron microscopy, viral particles of coronavirus were found in type II pneumocytes, and cylindrical epithelia from the bronchioles [13], which correlates with the cytopathic changes found with H&E.

In our cases, we observed markedly congested vessels (Figure 1), and prominent DAD with coexistence of exudative and proliferative phases in most patients

(Figure 2 and Figure 3). In the periphery of the more compromised areas, the presence of alveolar microthrombi was striking. The immunohistochemistry with TTF-1 highlighted the hyperplasia of atypical type II pneumocytes (Figure 4); and with CK7 collapsed alveoli were surrounded by a fibroblastic proliferation (Figure 5). As it was observed by others, we found scant inflammatory infiltrates in the majority of cases,

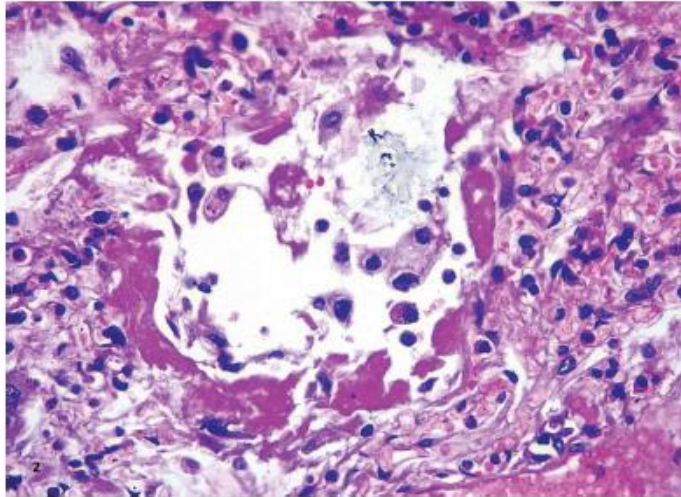


Figure 4: Hyaline membranes with atypical pneumocytes (HE,40X).

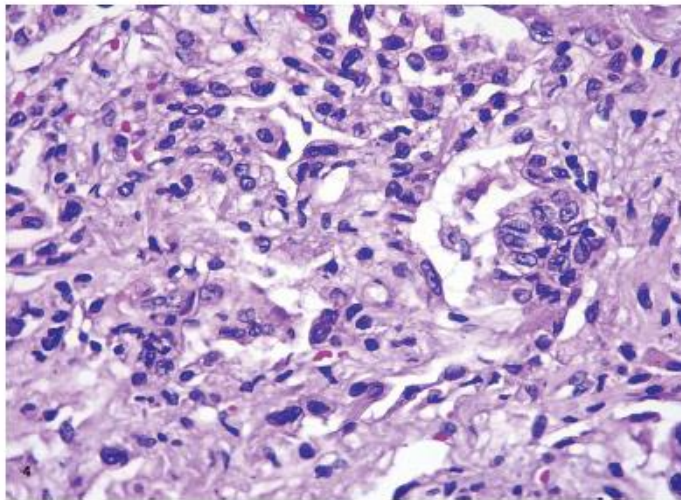


Figure 5: DAD early proliferative phase (HE,40X).

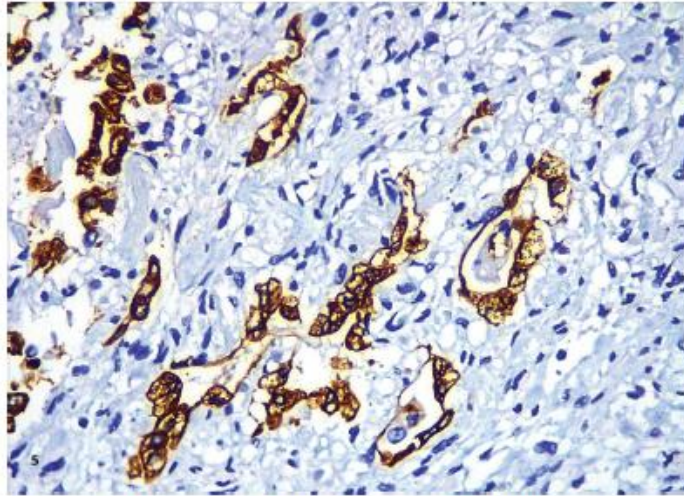


Figure 6: CK7 in an area of proliferative phase of DAD (40X).

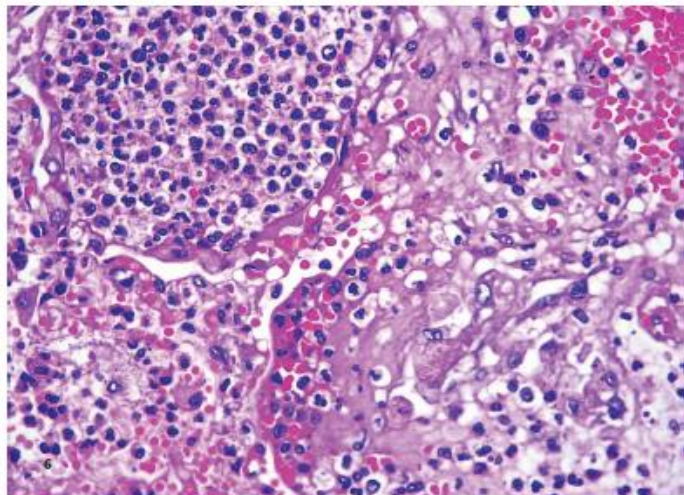


Figure 7: Neutrophilic exudate within alveoli in a patient complicated with bronchopneumonia (HE,40X).

but those complicated by bronchopneumonia (Figure 6). We also found giant multinucleated cells, which were positive to CK7, and had cytopathic-like changes in the majority of cases (Figure 7). The cytopathic-like changes consisted of large nuclei, nucleolar inclusions, lumpy chromatin, and a large, eosinophilic cytoplasm; however, these changes are not specific for Sars-cov-2, therefore they cannot be used as diagnostic criteria

for COVID-19 pneumonia. Variable amounts of CD68 positive alveolar macrophages were identified (Figure 8). Interestingly, higher amounts of alveolar macrophages were observed in patients with the highest values of ferritin (observation not systematized). This finding correlates with the role of ferritin in macrophage activation and the proposal to include COVID-19 within the hyperferritinemic syndromes [38].

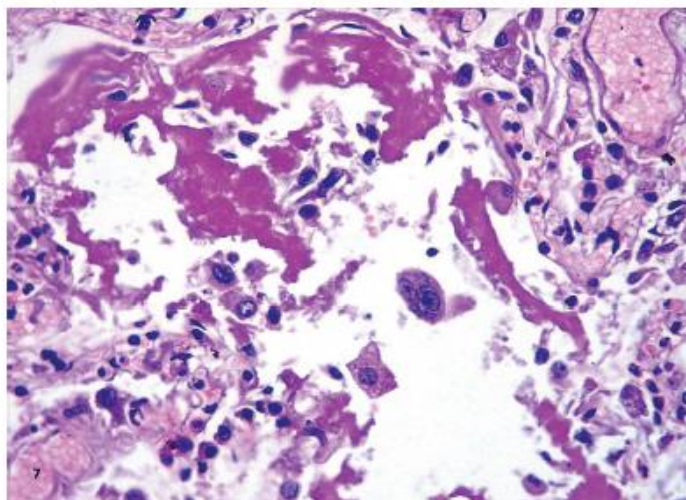


Figure 8: Hyaline membranes and a multinucleate atypical cell (HE,40X).

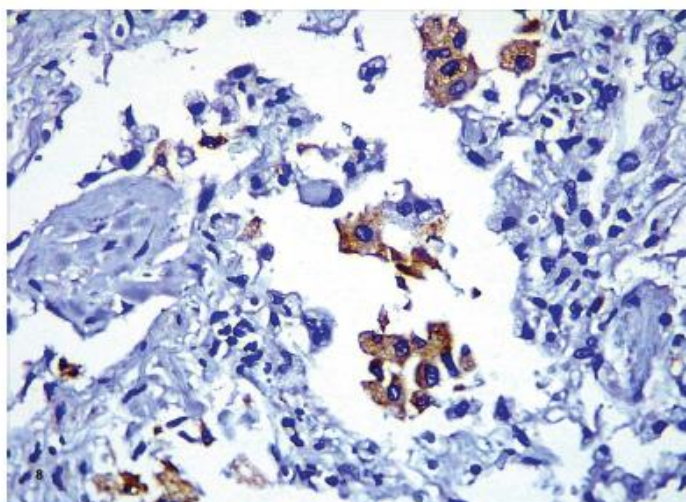


Figure 9: CD68 depicting alveolar macrophages (40X).

The immunohistochemistry with CD61 showed platelets and megakaryocytes forming aggregates within vessels in the alveolar septa (Figure 9), similar to the findings by Dohnikoff, Carsana and Fox [22,24,31]. We found large megakaryocytes inside vessels, which was not considered of diagnostic importance, as the lungs are a reservoir of these cells [39].

The most common finding in heart tissue our heart sample was aggregates of large brown vacuoles filled with lipofuscin, located at the poles of the nuclei in the cardiomyocytes (Figure 10 and Figure 11). Deposition of lipofuscin is considered normal as a part of the aging process [39]; however, it calls to our attention that the amounts observed surpass what is expected

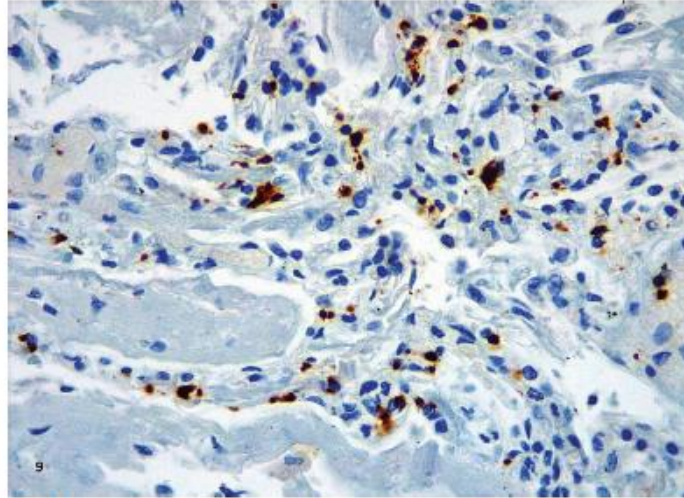


Figure 10: CD61 showing megakaryocytes (large dots) and aggregates of platelets (small dots) (40X).

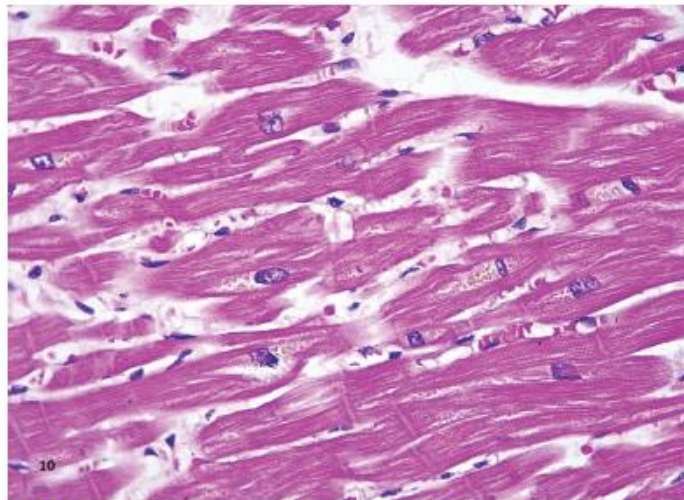


Figure 11: Section of the heart showing deposits of a paranuclear light-brown material (HE,40X).

in aging organs, especially in our six patients younger than 60-years-old. We hypothesize that the origin of these deposits is related to the cardiotoxicity of hydroxychloroquine, as all the patients in our study received cumulative doses from 0.8 to 7.6 g (400 mg/day) of hydroxychloroquine. Our dose is similar to the one used by Lane, et al. who showed an increased risk of heart disease in hydroxychloroquine users [40]. The

accumulation of lysosomes with lipofuscin has been previously reported in two patients with cardiac toxicity due to hydroxychloroquine, but the dose was not specified [41].

Other findings associated to chronic toxicity of chloroquine (cytoplasmic vacuolization with inclusions of PAS positive granular material), were not identified in our cases. It is important to point out that none of

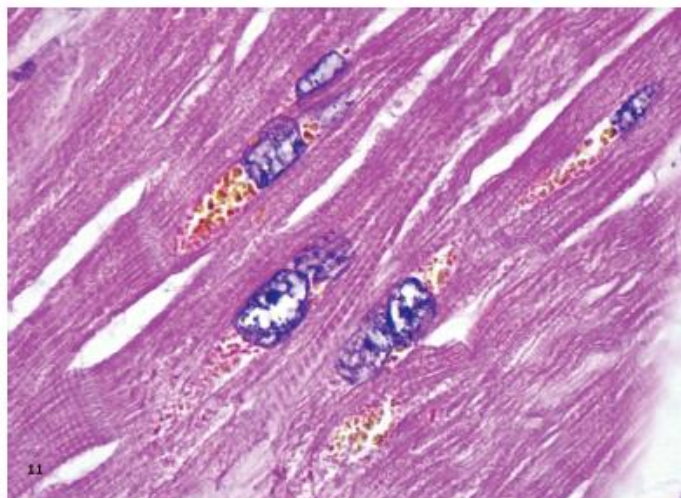


Figure 12: Another heart with similar light-brown aggregate (HE,100X).

the patients died as a result of cardiac dysfunction. Also, no significant electrocardiographically alterations were observed in any of the patients during hospitalization, even in the patient with myocardial microabscesses.

Other possible cause of lipofuscin in the heart is the oxidative damage caused by SARS-CoV-2 virus in multiple organs. S protein in the viral capsule induces the production of reactive oxygen species (ROS) through stimulation of NADPH oxidase [42]. Neutrophils are another source of ROS in covid-19 infection, especially in earlier stages of the disease [43]. These cells have been identified in myocardium tissue from infected patients [12,23,31], which would suggest that the heart is a target of the viral infection.

The findings in the liver, such as steatosis, sinusoidal dilatation and congestion (Figure 12), were found in the majority of cases. Mild inflammation was found in some cases and could be associated to preexisting comorbidities, drug effects, and/or unspecific systemic changes, as it has been pointed out by other authors [15,23].

In summary, our findings corroborate the histological injury in the lungs as previously reported. A larger number of alveolar macrophages were found in the cases with the highest values of ferritin. We also found aggregates of lipofuscin in all the hearts examined, probably in association with therapy with hydroxychloroquine.

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II. DISCUSIÓN

La recolección de las muestras para este estudio fue realizada durante la primera oleada de la epidemia de COVID-19, por iniciativa de uno de los autores del artículo. La idea original fue documentar el daño tisular producido en pacientes víctimas de la infección, en un contexto de emergencia sanitaria y falta de información sobre mecanismos y tipo de daño tisular asociados a la infección por este virus. Finalmente, este resultó ser el único estudio peruano de serie de casos documentando la histopatología de la infección por COVID-19.

El estudio realizado permitió confirmar la severidad del daño pulmonar asociado a esta infección sistémica, cuyo órgano blanco principal es el pulmón. La injuria pulmonar aguda (IPA) o síndrome de distrés respiratorio agudo del adulto (SDRAA) fue formalmente descrita por primera vez el año 1967 por Ashbaugh (1) como un síndrome caracterizado por disnea, reducción de la compliance pulmonar, infiltrados difusos en la radiografía de tórax e hipoxemia, sin respuesta a suplementos de oxígeno. Desde su descripción inicial, ha habido cambios en la clasificación de IPA, utilizándose desde el año 2012 la clasificación de Berlin, que gradúa la severidad de la hipoxemia como leve, moderada y severa (2).

En la clasificación etiológica de la IPA, se identifican causas infecciosas, siendo cualquier patógeno capaz de producir este tipo de daño en pacientes inmunocomprometidos; mientras que en pacientes inmunocompetentes, las causas infecciosas reconocidas con mayor frecuencia, antes de la pandemia de COVID-19

eran principalmente virales, incluyendo adenovirus, virus de influenza, citomegalovirus, hantavirus y virus sincitial respiratorio (3)

La patogenia de la injuria pulmonar aguda, independientemente de su etiología, incluye daño endotelial y daño alveolar, con formación de depósitos de material fibrinoide en la superficie alveolar, que reciben el nombre de membranas hialinas y que representan el daño tisular característico de la fase aguda de la IPA y que se denomina daño alveolar difuso (DAD). Luego de la fase aguda se describe una fase intermedia o reparativa y posteriormente una fase tardía u organizativa, cada una con sus alteraciones patológicas características. (3)

En algunos casos de IPA de origen infeccioso se pueden observar cambios citopáticos característicos, como por ejemplo células multinucleadas, con inclusiones virales intracitoplasmáticas e intranucleares a nivel intersticial y alveolar en casos de sarampión. (4) En infección por adenovirus se han descrito dos tipos de inclusiones virales, una inclusión eosinofílica intranuclear, rodeada de un halo y una célula “manchada”, de tamaño grande, citoplasma basofílico, sin inclusiones ni halos. (5) Se puede observar multinucleación y moldeamiento nuclear con núcleos en “vidrio esmerilado” en infecciones por herpesvirus y por virus de varicela zoster; mientras que inclusiones eosinofílicas prominentes y aumento de tamaño nuclear, caracterizan a la infección por citomegalovirus; sin embargo, estas infecciones son raras en pacientes inmunocompetentes. (2) En IPAs causadas por otros virus, como virus de influenza o hanta virus no se han descrito cambios citopáticos característicos. (2)

Cuando se describieron las primeras alteraciones patológicas en pulmones de pacientes infectados por COVID-19 hubo un interés por demostrar si existía un efecto citopático, que sea distintivo o característico. En todas las publicaciones que incluyeron una descripción detallada del daño histopatológico en pacientes con COVID-19 se vio que existía coincidencia, en señalar que no se observaron cambios citopáticos que sean característicos de daño pulmonar asociado a COVID-19, (6,7) señalándose incluso que el daño alveolar difuso en COVID-19 es indistinguible del ocasionado por otros factores etiológicos. (6) En la presente serie de casos, los pulmones mostraron membranas hialinas (Figura 1), vasos marcadamente congestivos, microtrombos, hiperplasia de neumocitos, escaso infiltrado inflamatorio, ocasionales células gigantes multinucleadas y neumocitos con efecto citopático, caracterizado por núcleos grandes, inclusiones nucleares (Figura 2), cromatina grumosa y citoplasma amplio y eosinofílico, aunque se enfatizó que el efecto citopático no podría considerarse distintivo. Casos más avanzados de daño mostraron expansión estromal con alveolos colapsados, mejor demostrados con la inmunohistoquímica para Citoqueratina 7 (Figura 3), característicos de la fase proliferativa/organizativa del daño alveolar difuso.

Los dos hallazgos más relevantes a nivel pulmonar, en consonancia con la literatura publicada, fueron la presencia de agregados de plaquetas, resaltados con la inmunohistoquímica con CD61, en los vasos de los septos alveolar y la presencia de macrófagos alveolares, positivos a CD68, más numerosos en los casos de pacientes con valores más elevados de ferritina. El curso clínico catastrófico de los casos de COVID-19 ha sido asociado a un daño endotelial severo y extenso, no

limitado a los pulmones, que es responsable de disfunción generalizada, cuya traducción histológica consiste en endotelitis, dilataciones vasculares y fenómenos microtrombóticos en la mayor parte de casos y trombosis de vasos más grandes en unos pocos casos. (7-10) En la patogenia del COVID-19, estos procesos se han asociado a un concepto denominado Trampas Extracelulares de Neutrófilos (NETosis), asociado a la activación de la cascada de la coagulación, como parte de un fenómeno de inmutrombosis. (8,9) Es interesante resaltar que la presencia de microtrombos ha sido más frecuentemente reportada en las series de casos publicadas en el año 2020, siendo menos frecuente en las series de casos del 2021, lo que podría reflejar cambios en la terapia, que incluyó el uso de anticoagulantes en los casos más recientes. (11) Al respecto, cabe mencionar que las muestras que conforman la presente serie de casos, fueron tomadas entre abril y mayo del año 2020, en los inicios de la pandemia. Por otro lado, desde los inicios de la pandemia se reconoció un severo compromiso sistémico en pacientes con COVID-19, con niveles muy elevados de proteína C reactiva y de ferritina en sangre, coagulopatía, linfopenia y daño hepático, característicos de un síndrome hiperinflamatorio, previamente descrito en niños con Enfermedad de Still (artritis reumatoidea juvenil), bajo la denominación de síndrome de activación macrofágica o linfocitosis hemofagocítica secundaria. (12) En los síndromes hiperferritinémicos, se ha señalado al hígado y a los macrófagos activados como fuente de ferritina, que no solo sería un marcador de la inflamación, sino que también sería un indicador de activación macrofágica. (13) Desde los primeros reportes de la relación entre los niveles séricos elevados de ferritina y la severidad de COVID-19, (14) todas las publicaciones posteriores confirmaron esta

observación. Una contribución de este estudio es que se observa que la cantidad de macrófagos alveolares fue mayor en los pacientes con niveles más elevados de ferritina.

Como enfermedad sistémica, la infección por COVID-19 tiene como uno de sus órganos blanco más importantes, después del pulmón, al corazón. El espectro de las manifestaciones cardíacas asociadas a infección por COVID-19 incluye: arritmias, trastornos isquémicos, disfunción ventricular, insuficiencia cardíaca descompensada, shock cardiogénico, miopericarditis y miocarditis. (15) En una revisión sistemática de autopsias se ha estimado en un 8% la frecuencia de miocarditis asociada a COVID-19; sin embargo, el mismo estudio señala que, de aplicarse los criterios de Dallas o de Marbourg para el diagnóstico de miocarditis, solo un 1.4% de los casos publicados cumpliría los requisitos diagnósticos; (16) sin embargo, hasta en un 47% de autopsias de pacientes fallecidos con COVID-19 se ha podido observar evidencia histológica de daño caracterizado por infiltrados inflamatorios, trombos microvasculares, necrosis focal y daño aleatorio de cardiomiocitos, hasta en un 47%. (15,16)

Un metaanálisis que incluyó 177 casos en 23 publicaciones, reportó hipertrofia miocárdica en (51.2%), fibrosis miocárdica (50%), alteraciones de pequeños vasos coronarios (25.9%), infiltrados inflamatorios miocárdios (15.9%), amiloidosis (5.9%) y necrosis (5.3%). (17) En la presente serie de casos no hubo evidencia clínica documentada de daño miocárdico; sin embargo, en 11 de los 18 casos se pudo evidenciar infiltrado inflamatorio a nivel del miocardio, lo que concuerda con

la literatura revisada. A diferencia de toda la literatura revisada, en los casos que se presentan, el hallazgo de depósitos de lipofuscina en todas las muestras evaluadas (Figura 4), no tiene precedentes y, luego de una revisión extensa de la literatura, se propone que este hallazgo guardaría relación con el uso combinado de antimaláricos como cloroquina y macrólidos como azitromicina, ambos con potencial cardiotoxicidad, al inicio de la pandemia; sin embargo, es muy probable que este hallazgo no tenga mayor relevancia, debido a que no se llegó a documentar falla miocárdica, en ninguno de los casos. La lipofuscina es un pigmento que se acumula en los lisosomas de las células senescentes como consecuencia de la degradación de proteínas que sufren daño oxidativo. (18) Se considera que existe una considerable variación en los constituyentes de la lipofuscina en diferentes regiones corporales; sin embargo, se estima que sus componentes principales son proteínas y lípidos oxidados con uniones cruzadas, pequeñas cantidades de carbohidratos y algunos metales como hierro, calcio, zinc, cobre y manganeso. (19) En los inicios de la pandemia se consideró que, actuando como una base débil, la hidroxiclороquina incrementaría el pH de las vesículas endosómicas intracelulares, contribuyendo a disminuir la carga viral; (20) sin embargo, un metaanálisis que incluyó 19 estudios con 5652 pacientes estimó que la frecuencia de “torsade de pointes”, taquicardias ventriculares y paros cardiacos fue de 3 por 1000. (21) Un estudio realizado en ratas que recibieron el equivalente a dosis terapéuticas de hidroxiclороquina demostró ondulación, desorden, desorganización y ocasional necrosis de miofibrillas cardiacas. (22) Finalmente, un estudio más reciente, evaluó la relación entre lipofuscina, envejecimiento de miofibrillas cardiacas y autofagia, determinando que la lipofuscina ejerce un efecto de declive en la función cardiaca,

que la acumulación de este pigmento está directamente relacionado con mecanismos de autofagia que se activan con la senescencia y que, curiosamente, la cloroquina funcionaría como un inhibidor de la autofagia, al suprimir la fusión de autofagosomas con lisosomas e incrementar el pH lisosomal. (23) Otro estudio, realizado antes de la pandemia de COVID-19, evaluó la cardiotoxicidad de la cloroquina, reportó el hallazgo de vacuolas citoplasmáticas con inclusiones de material granular positivo a la tinción de PAS, que dan la apariencia de desorganización de miofibrillas. El estudio ultraestructural demostró que estas inclusiones corresponden a cuerpos curvilíneos de inclusión, característicos de la toxicidad por cloriquina. (24) Otras hipótesis del daño miocárdico en pacientes con COVID-19 incluyen injuria miocárdica directa por parte del virus y compromiso miocárdico, como parte de respuesta inflamatoria sistémica. (9)

Finalmente, en relación a la patología hepática asociada a COVID-19 en la presente serie, fueron observados infiltrados linfocíticos periportales en 4 de los casos y otros hallazgos menos frecuentes incluyeron dilataciones sinusoidales (Figura 5) y microtrombos. En una revisión de la patología hepática de 21 artículos publicados que incluían hallazgos en 118 casos, se observó esteatosis (59.3%), fibrosis (31.1%), congestión vascular (29.6%), infiltrados inflamatorios (27.1%), necrosis (22.1%), colestasis (4%) y cirrosis (2%). (17) Estos hallazgos, permiten estimar que, a pesar de ser una fuente importante de moléculas asociadas a la respuesta inflamatoria en pacientes con COVID-19, el hígado parece ser un órgano que evidencia menor frecuencia de injuria celular por esta enfermedad.

En conclusión, este estudio representa la única serie de casos peruana de cambios patológicos asociados a infección por COVID-19, con hallazgos que permiten: 1) Confirmar la severidad de la injuria pulmonar; 2) Definir la ausencia de cambios citopáticos que se pudieran considerar característicos de la infección; 3) Resaltar el rol de los macrófagos (activación macrofágica) y las plaquetas (fenómenos microtrombóticos) como parte de una respuesta inflamatoria severa; 4) Corroborar la presencia de injuria cardiaca, con el hallazgo de depósitos intracelulares de lipofucsina cuyo origen queda por ser aclarado.

CONCLUSIONES Y RECOMENDACIONES

Este estudio representa la única serie de casos peruana de cambios patológicos asociados a infección por COVID-19, con hallazgos que permiten:

1. Confirmar la severidad de la injuria pulmonar
2. Definir la ausencia de cambios citopáticos que se pudieran considerar característicos de la infección
3. Resaltar el rol de los macrófagos (activación macrofágica) y las plaquetas (fenómenos microtrombóticos) como parte de una respuesta inflamatoria severa
4. Corroborar la presencia de injuria cardíaca, con el hallazgo de depósitos intracelulares de lipofucsina cuyo origen queda por ser aclarado.

La pandemia del COVID-19 representó un enorme desafío para los sistemas de salud a nivel global y constituyó una oportunidad excepcional para el desarrollo de investigaciones que contribuyeron a conocer mejor la patogenia de la enfermedad, y que fueron realizadas desde el inicio de la pandemia. No hubo una preparación previa para un escenario de esta naturaleza y, en base a la experiencia adquirida, la recomendación sería que tanto los equipos asistenciales como los de investigación estén mejor preparados para una respuesta más rápida y efectiva, con el apoyo del sistema de salud.

III. REFERENCIAS BIBLIOGRÁFICAS

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FOTOS MICROSCÓPICAS

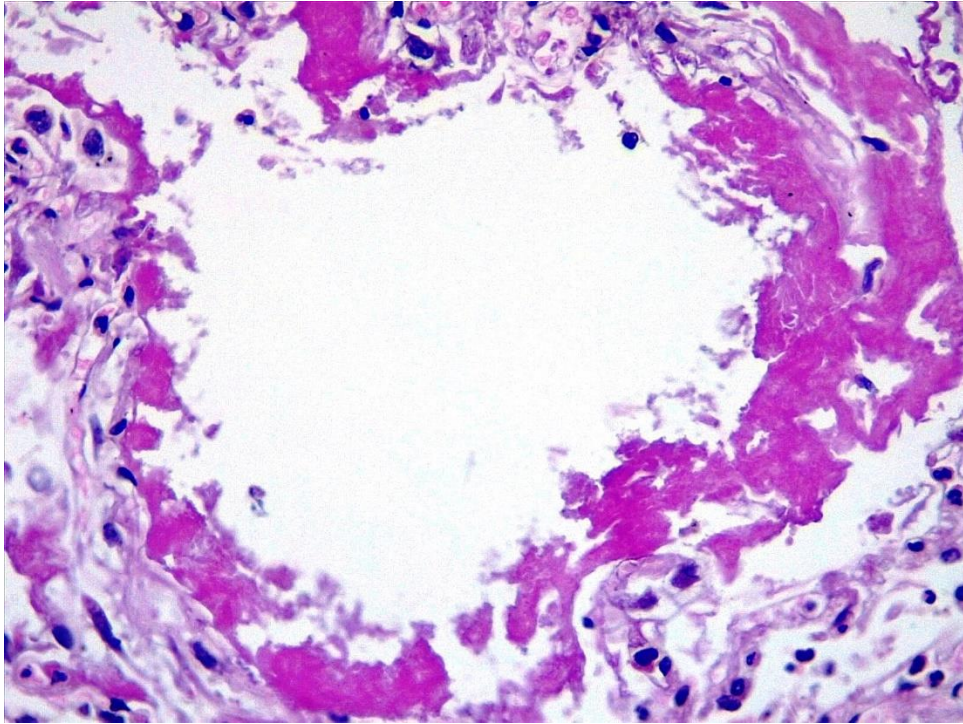


Figura 1. Membranas hialinas tapizando la superficie alveolar, evidencia histológica de daño alveolar difuso. H&E 40X

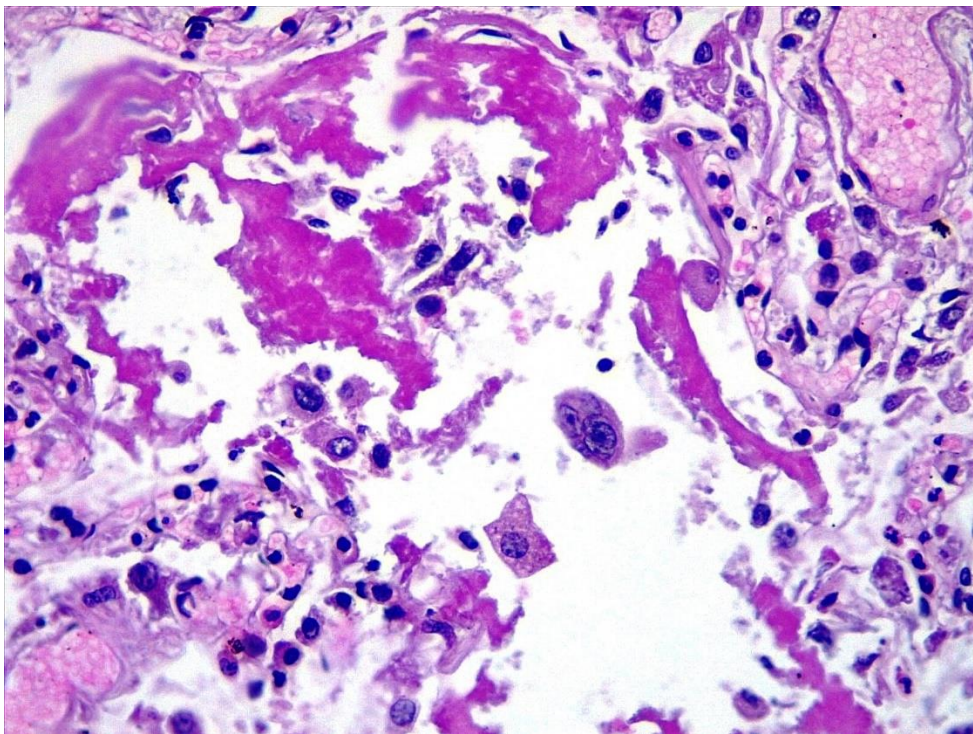


Figura 2. Una célula multinucleada a nivel intraalveolar, membranas hialinas y neumocitos de núcleos grandes con nucléolos visibles. H&E 40X

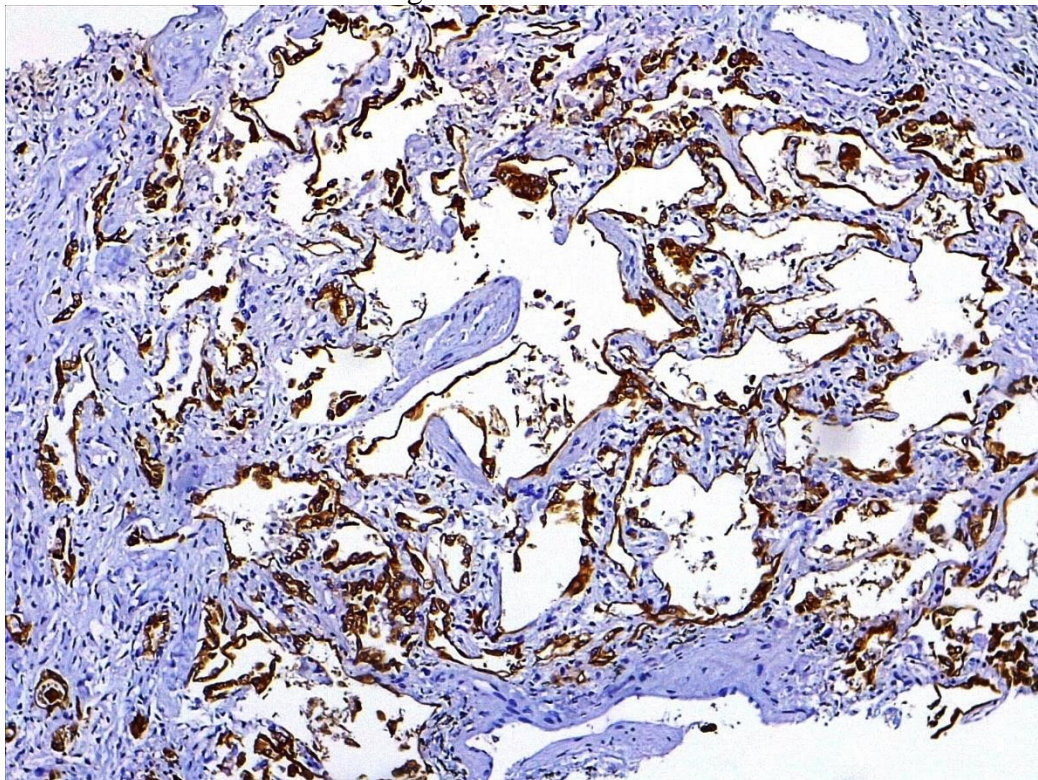


Figura 3. DAD en fase organizativa/reparativa, con engrosamiento de septos alveolares y escaso infiltrado inflamatorio agudo. La inmunohistoquímica con citoqueratina 7 colorea los macrófagos alveolares. (40X)

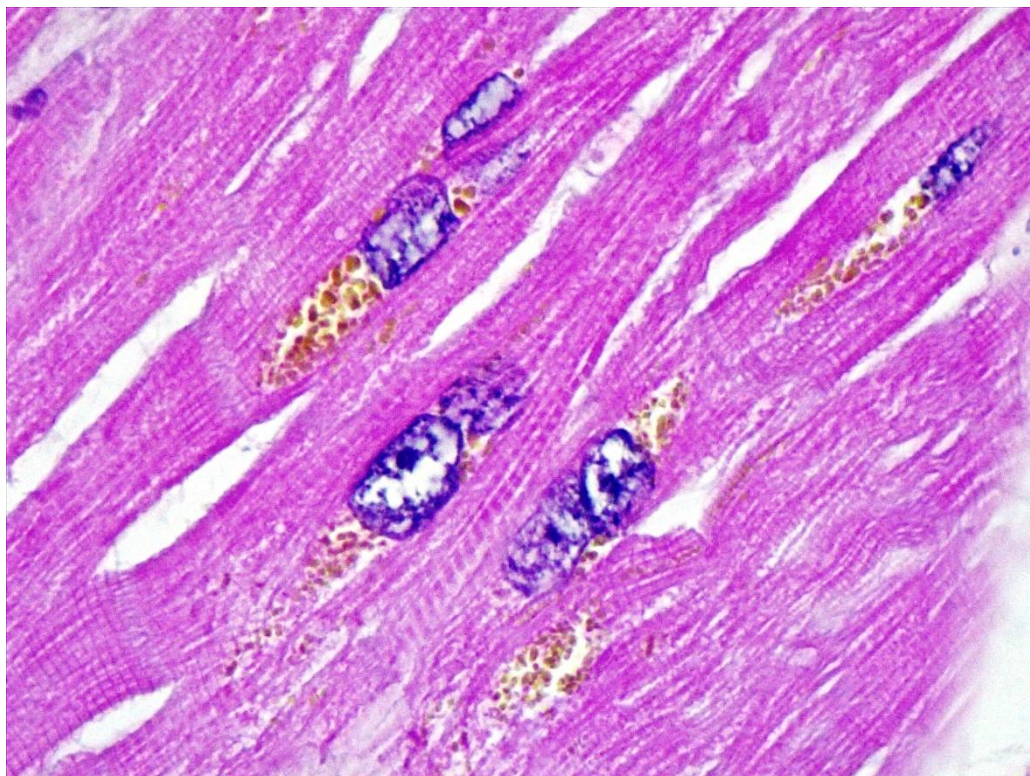


Figura 4. HE100X. Depósitos de lipofucsina a nivel paranuclear en el miocardio

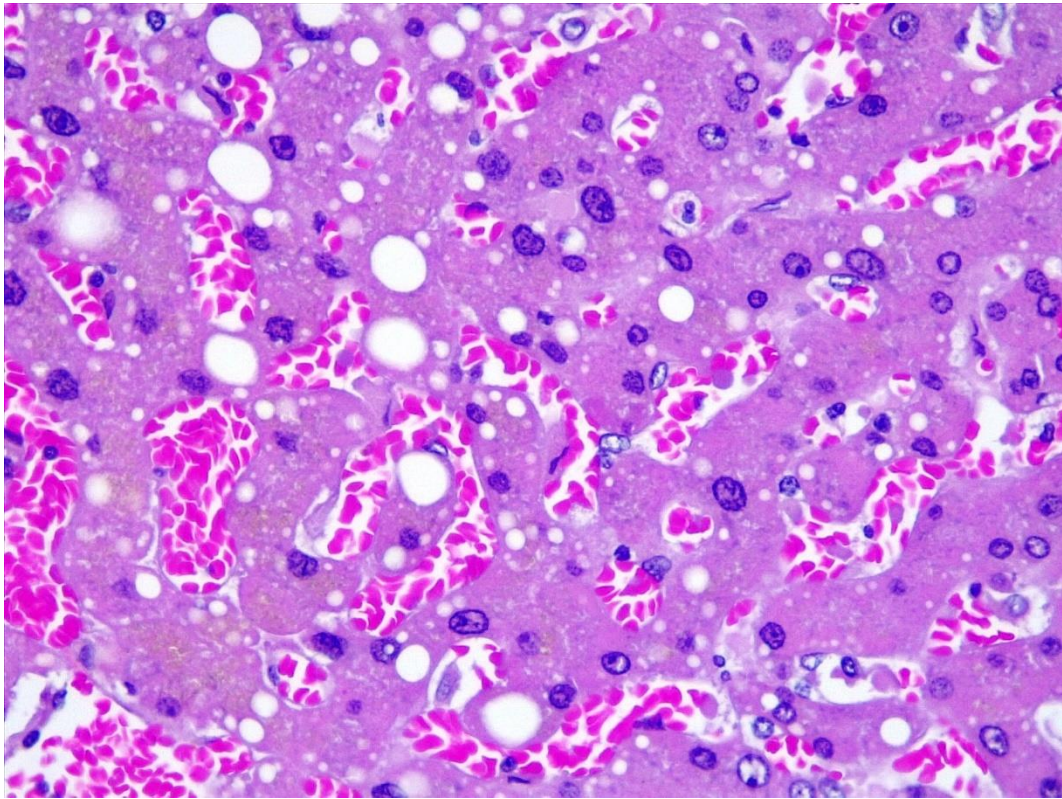



Figura 5. H&E 40x. Tejido hepático con marcada congestión vascular sinusoidal

IV. ANEXOS

 UNIVERSIDAD PERUANA CAYETANO HEREDIA	UNIDAD DE GOBIERNO Y ADMINISTRACIÓN	
	SISTEMA DE ASEGURAMIENTO DE LA CALIDAD	V.01.04 / 27.09.2023
	MANUAL OPERATIVO DE LAS NORMAS Y PROCEDIMIENTOS DEL TRABAJO DE INVESTIGACIÓN PARA LA OBTENCIÓN DE LOS GRADOS ACADÉMICOS DE MAESTRÍA Y DOCTORADO	ESCUELA DE POSGRADO VÍCTOR ALZAMORA CASTRO
		Página 1 de 30

a. Cuando el estudiante no es el primer autor del artículo.

Documento de respaldo del Investigador Principal

Estimados Sres. Escuela de Posgrado Víctor Alzamora Castro

Me dirijo a usted en calidad de AUTOR PRINCIPAL del artículo de investigación titulado "PATHOLOGICAL CHANGES IN POSTMORTEM COVID-19 BIOPSIES", de la autoría del estudiante César Augusto Chian García.

Deseo expresar mi respaldo de la información presentada en la declaración jurada del estudiante con respecto al desarrollo temático del artículo de investigación. Confirmando que el estudiante es el autor secundario del artículo de investigación y su participación consistió en:

- Realizar una revisión sistemática de la literatura sobre el tema
- Ordenar y evaluar las pruebas de inmunohistoquímica realizadas a las muestras
- Tomar las microfotografías de las biopsias
- Elaborar las tablas con los resultados
- Elaborar el primer borrador de la publicación

Además, puedo afirmar que los datos e información incluidos en el artículo de investigación adjunto se basan en una metodología rigurosa y una revisión crítica de la literatura relevante. Asimismo, puedo atestiguar que el estudiante ha cumplido con todas las normas éticas y de integridad académica en la realización de este artículo, y que la información presentada es precisa y confiable. Agradezco la oportunidad de respaldar la información presentada en la declaración jurada del estudiante, y me pongo a su disposición para cualquier consulta adicional que pueda tener.

Atentamente,



Dr. Alejandra Guerrero
Departamento de Patología
Centro Médico Naval