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Cytopathological Findings in Bronchoalveolar Lavage from Patients with COVID-19

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Keywords
COVID-19 · Bronchoalveolar lavage · Cytopathological finding

Abstract
Information on cellular analysis of bronchoalveolar lavage (BAL) in patients with COVID-19 is limited. Some studies have described an increase in lymphocyte percentage or exuberant plasmacytosis. Some reports addressed the importance of molecular testing on BAL samples to confirm COVID-19 pneumonia, in clinically highly suspected patients with consecutive negative nasopharyngeal swab results. In addition to atypical lymphocytes in the peripheral blood, morphologic findings of atypical lymphocytes in BAL were also reported in a few patients. The objective of this study was to describe the cytopathic characteristics identified, any data presented here are descriptives and intended to trigger further research. Three general aspects have been evaluated in each sample: reactive changes, virus-related pathological changes, and differential leukocyte count. Seventeen samples were collected. All samples were negative for malignancy, with an inflammatory background, predominantly lymphohistiocytic in 5 samples, histiocytic in 9, and 3 with predominantly neutrophilic. Hemosiderin-laden macrophages were observed in 12/17. Nonspecific reactive cell changes were identified in 4 samples, including bronchial, alveolar, and reserve cell hyperplasia. Virus-related pathological changes were observed in 14 samples, such as loss of nuclear chromatin pattern, lymphocytes with atypical nuclei, nuclear and cytoplasmic inclusions, multinucleations in bronchial cells and macrophages, or multinucleated giant cells. The identification of multinucleated giant cells could represent a cytopathic effect induced by the virus, at the same time the nuclear clearance of pneumocytes as a possible direct effect. BAL is a procedure aimed at obtaining cells from the respiratory tract that can provide valuable and rapid information. It is important to collect and describe as many cytopathological findings as possible, which can provide relevant information for future studies.

Introduction

The new coronavirus, called *SARS-CoV-2*, was isolated for the first time in Wuhan, China, in December 2019 [1]. Its outbreak and the rapid worldwide spread of CO-

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VID-19 along with its multiple consequences meant the study of such pathology represents a global and emerging need at a scientific level.

Autopsies produced an organic overview of the COVID-19 landscape in the lungs. As a second step, pathologists are translating the histological information into mini-invasive samples such as bronchoalveolar lavage (BAL), which might play a significant role in the multidisciplinary workup of *SARS-CoV-2* infection [2, 3].

Performing BAL with the flexible bronchoscope is a simple, safe, well-tolerated technique that provides valuable clinical information in the study of various lung diseases. When this technique was first described in the late sixties, such a significant and growing development over the years was never expected from it. The application of BAL in the microbiological study became popular in the 1980s with the emergence of the AIDS epidemic and in the 1990s for the study of pneumonia associated with mechanical ventilation [4].

The technique consists of instilling saline in 20–50 mL boluses up to the desired total volume through the internal channel of the bronchofiberscope, after fitting it into the chosen bronchus. After each instillation, it is aspirated with the same syringe, with adequate pressure, so as not to collapse the bronchial walls [5].

Among the indications for BAL, one of the most frequent is the diagnosis of virus infections. Unlike pneumonia due to fungi or bacteria, viral infections often induce specific cytopathic changes that allow the pathologist to make a firm diagnosis of the causative agent. This is particularly important since other methods may not be available, less profitable, or not as accurate.

Cytopathic findings can be nonspecific or specific for viral infection [6]. Among the nonspecific, exudation of inflammatory cells and necrotic debris can be seen in early stages of *influenza* or *parainfluenza* infections. Degenerative changes can be identified as remnants of fragmented hair cells of the bronchial columnar epithelium, with cytoplasmic swelling and nuclear pyknosis. This phenomenon was described for the first time in *adenovirus* infections. In addition, hyperplasia of bronchial and alveolar cells can be observed, arranged in clusters, with swollen hyperchromatic nuclei and prominent nucleoli.

Specific changes for certain viruses have been described and well recognized, such as intranuclear inclusion bodies, loss of the nuclear chromatin pattern, multinucleations, or cytoplasmic inclusions. These changes can be seen mainly in infections by *herpes virus*, *varicella zoster*, *cytomegalovirus*, *respiratory syncytial virus*, or *adenovirus*.

Studies based on findings presented in BAL patients with various pulmonary pathologies have been increasing in recent years due to their cost-effectiveness and the wide sampling source they represent. BAL has become a useful bronchoscopic medium for sampling the cells of the respiratory tract, soluble products, and pathogens that line the alveolar spaces [7]. All this, in numerous pathologies, including the current COVID-19 disease.

Information on cellular analysis of BAL and its clinical significance in patients with COVID-19 is limited. Two earlier single case reports have described an increase in lymphocyte percentage or exuberant plasmacytosis in BAL fluid in patients with severe COVID-19 [2, 8]. A study of 20 patients reported BAL lymphocytosis with plasmacytosis [9].

Some reports addressed the importance of molecular testing on BAL samples to confirm COVID-19 pneumonia in clinically highly suspected patients with consecutive negative nasopharyngeal swab results [10–12]. Others described increased lymphocyte percentage or exuberant plasmacytosis in BAL fluid in 2 patients [8, 9]. In addition to atypical lymphocytes in the peripheral blood, morphologic findings of atypical lymphocytes in BAL fluid were also reported in a few patients [13–15].

The objective of this study was to describe the cytopathic characteristics identified in BAL samples from these patients. Any data presented here are descriptives and intended to trigger further research. In any case, previous reports suggest that BAL may be a proper specimen for in-depth diagnostics [16] and also the cytopathological observations may be confirmative factors for COVID-19 and could lead to *SARS-CoV-2* testing if not previously initiated.

Materials and Methods

This is a descriptive study, in which samples of BAL from patients affected by COVID-19 were studied. The samples that were sent by the Department of Pneumology of the Fundación Alcorcón University Hospital (Madrid-Spain), to the Department of Pathology of the same center, from May 2020 to May 2021, were included.

A BAL was present when meeting the Chamberlain criteria: degree of degeneration less than 20% in samples from cytocentrifuge, the liquid obtained had to be a minimum of 3 cc, observing a monolayer distribution of cells in the smear, and not having excessive blood contamination. Broken or incorrectly mounted sheets and material with external contaminants were excluded.

Personally identifiable information and demographic aspects obtained from the electronic record of medical records were collected. The procedure notes were also reviewed to assess the endoscopic description. Each sample was cytocentrifuged at 1,500 rpm

Table 1. Epidemiological characteristics, clinical history, and clinical outcome of the cases studied

Patient	Age, years	Sex	Clinical history	Presenting illness/symptoms	Clinical outcome
1	60	F	Rheumatoid arthritis, GERD, hiatal hernia	Cough, expectoration, dyspnea, and fever	Died
2	56	F	T2D, GERD, obese class 2, gonarthrosis	Cough, myalgia, and pleuritic chest pain	Alive
3	68	M	CKD, hypercholesterolemia, OSAHS	Cough, dyspnea, and fever	Alive
4	79	F	HT	Dyspnea and fever	Alive
5	72	F	SCH, Crohn's disease	Dyspnea and fever	Alive
6	31	M	Trisomy 21, interventricular communication, obese, OSAHS	Dyspnea and orthopnea	Alive
7	47	F	NHL, SLE	Cough, expectoration, and fever	Alive
8	62	M	Isoniazid-resistant tuberculosis	Headache and pleuritic chest pain	Alive
9	70	F	Breast cancer, SCH	Cough, dyspnea, and asthenia	Alive
10	56	M	*Pemphigus vulgaris*	Dyspnea, cough, and fever	Alive
11	79	M	HT, T2D, dyslipidemia	Cough and dyspnea	Alive
12	75	F	NHL, dyslipidemia	Sickness, fever, cough, and dyspnea	Alive
13	80	M	BPH	Cough, expectoration, and dyspnea	Alive
14	80	F	HT, T2D, dyslipidemia, breast cancer, NHL	Cough and dyspnea	Alive
15	54	F	Intermittent asthma	Dyspnea, asthenia, and pleuritic chest pain	Alive
16	52	F	No significant past medical history	Dyspnea, fever, and pleuritic chest pain	Alive

M, male; F, female; GERD, gastroesophageal reflux disease; T2D, type 2 diabetes; CKD, chronic kidney disease; OSAHS, obstructive sleep apnea-hypopnea syndrome; HT, hypertension; SCH, subclinical hypothyroidism; NHL, non-Hodgkin lymphoma; SLE, systemic lupus erythematosus; BPH, benign prostatic hyperplasia.

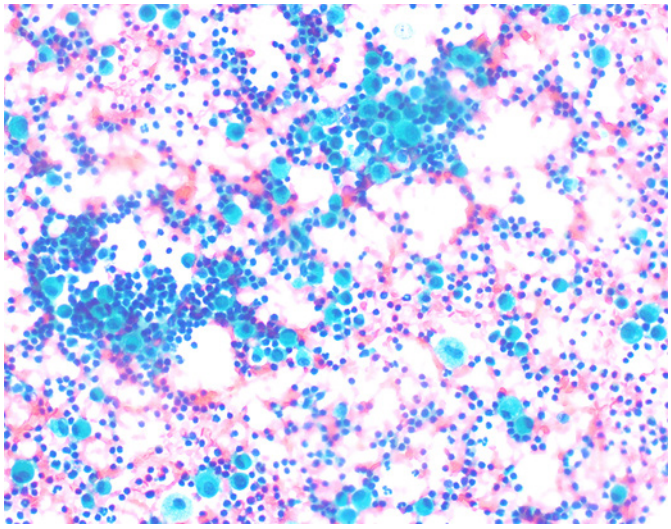

Fig. 1. Inflammatory background with a predominance of lymphocytes and histiocytes.

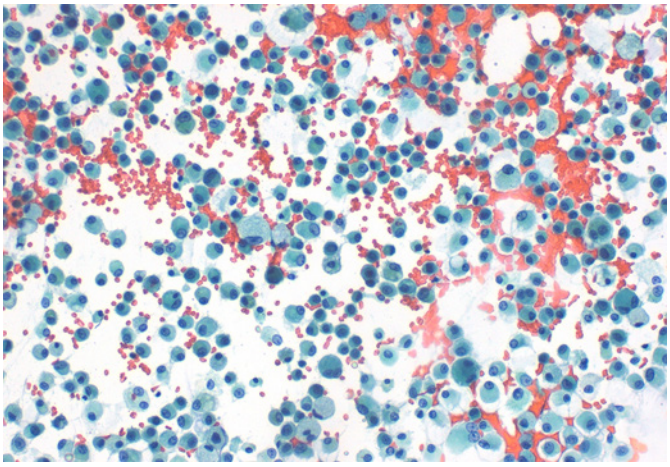

Fig. 2. Predominantly histiocytic inflammatory background.

for 10 min (Cytospin III, Shandon Instruments, Sewickley) and processed with Papanicolaou staining after fixation in 96% alcohol.

The cytological characteristics were reviewed and the relevant findings were evaluated by two pathologists, in different fields of low and high power under the light microscope, until the complete review of the sample and subsequent cell count in a high-power field (×400). Three general aspects have been evaluated in each sample: reactive changes, virus-related pathological changes, and differential leukocyte count.

As a reactive change, we looked at hyperplasia of bronchial and alveolar cells, metaplasia, degeneration, repair, or inflammation. We also looked for virus-related pathological changes, such as intranuclear inclusion bodies, loss of the nuclear chromatin pattern, multinucleations, and cytoplasmic inclusions. Later, the differential leukocyte count for neutrophils, lymphocytes, eosinophils, and histiocytes was determined.

Data analysis was performed with Excel version 16.16.27. Qualitative variables were analyzed with absolute and relative frequen-

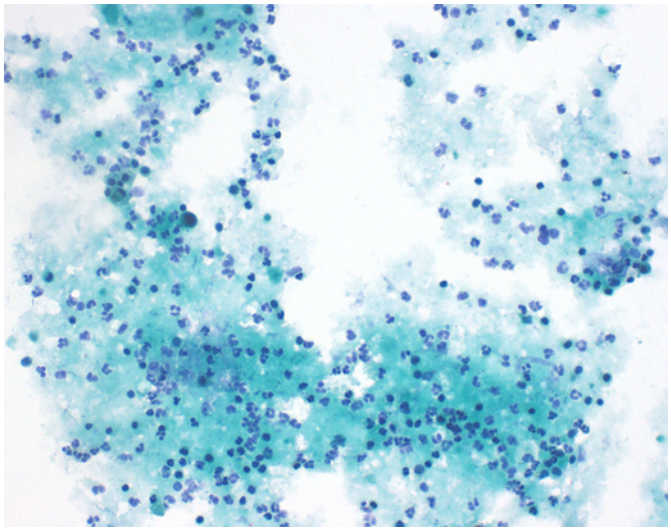

Fig. 3. Inflammatory background with acute inflammation.

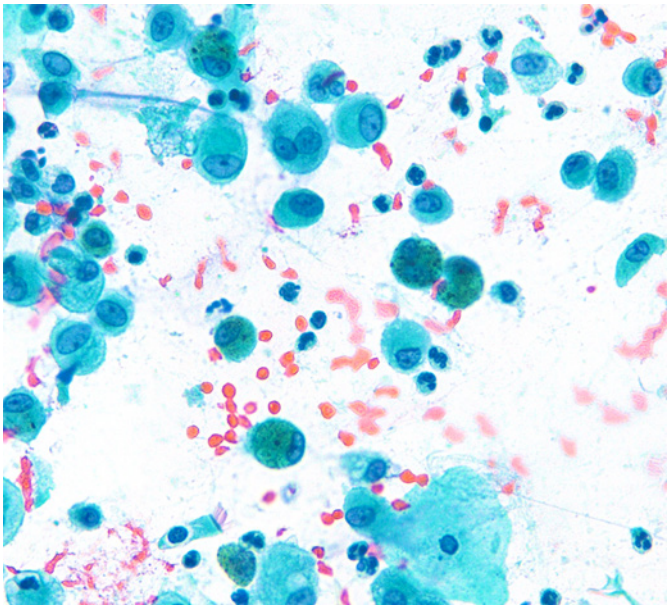

Fig. 4. Hemosiderin-laden macrophages.

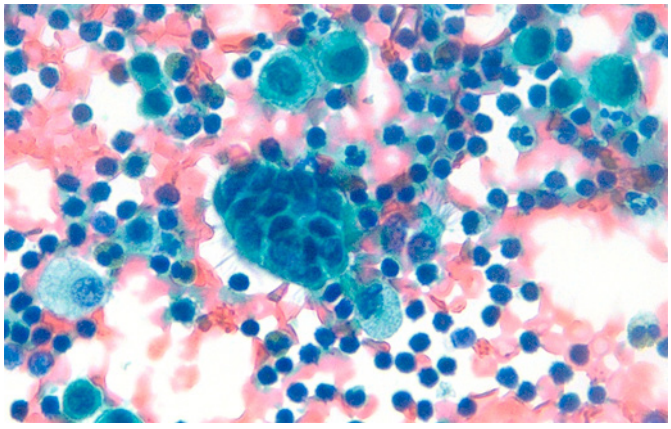

Fig. 5. Reserve cell hyperplasia.

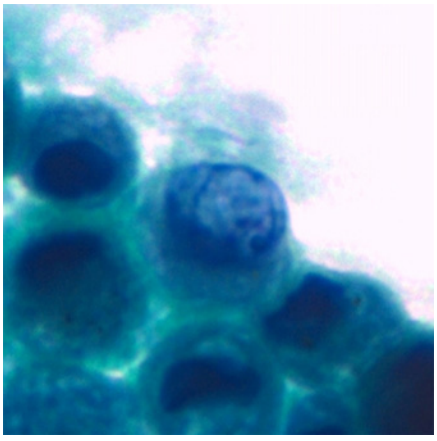

Fig. 6. Loss of nuclear chromatin pattern.

cies, and quantitative variables with measures of central tendency and dispersion. This study was approved by the committee and the informed consent was obtained from participants of this study.

Results

Sixteen patients who fit the study parameters were identified, of which a total of 17 BAL cytology samples were collected. The patients were between 31 and 80 years of age, with a mean of 62.8 ± 14.2, with 10 being female (62.5%) and 6 male (37.5%). Four patients (25%) had a known diagnosis of malignancy (3 with non-Hodgkin lymphoma, 2 with breast carcinoma). Only one case had a fatal outcome. Table 1 shows the demographic criteria, antecedents, symptoms, and clinical resolution of the cases studied.

On cytological analysis, all 17 samples were negative for malignancy, with an inflammatory background, predominantly lymphohistiocytic in 5 samples (shown in

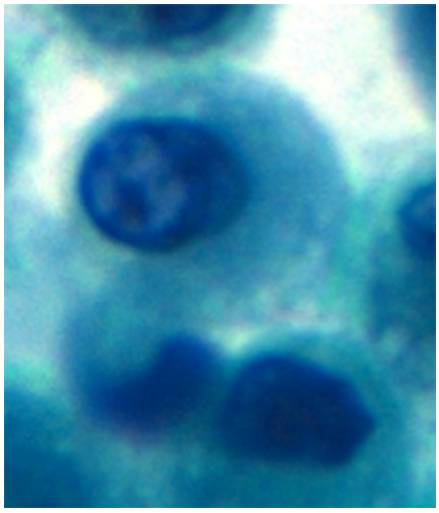

Fig. 7. Nuclear inclusions.

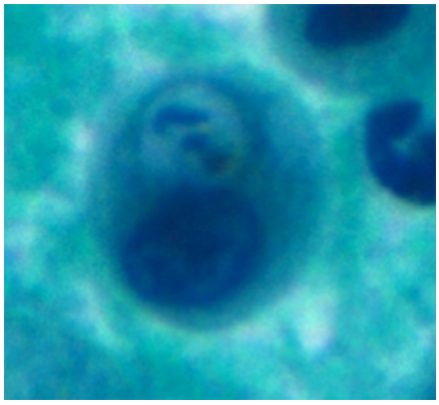

Fig. 8. Cytoplasmic inclusions.

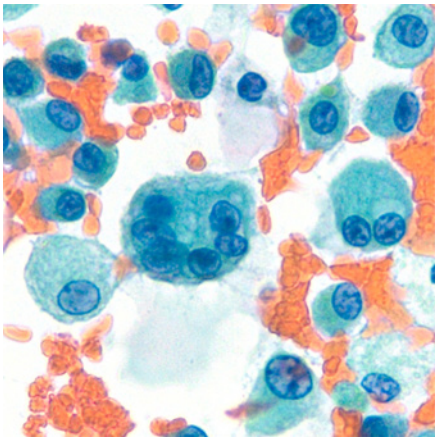

Fig. 9. Presence of multinucleated alveolar macrophages.

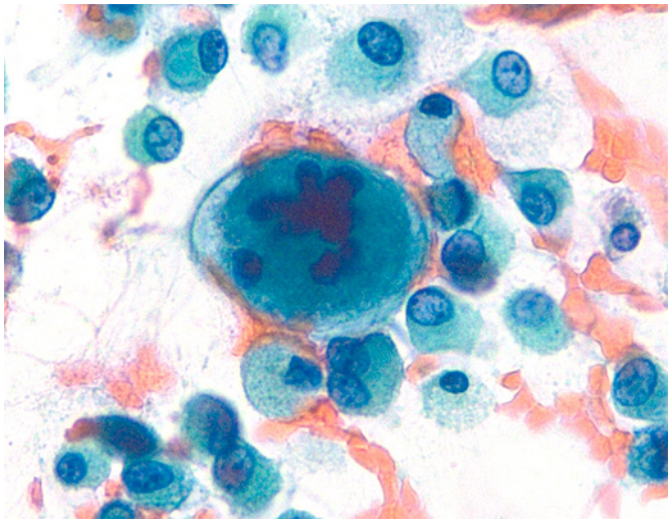

Fig. 10. Multinucleated giant cells.

Fig. 1), histiocytic in 9 (shown in Fig. 2), and 3 with predominantly neutrophilic (shown in Fig. 3). Hemosiderin-laden macrophages were observed in 12/17 samples (shown in Fig. 4).

Nonspecific reactive cell changes were identified in 4 samples, including bronchial, alveolar, and reserve cell hyperplasia (shown in Fig. 5). Virus-related pathological changes were observed in 14 samples, such as loss of nuclear chromatin pattern (shown in Fig. 6), lymphocytes with atypical nuclei, nuclear and cytoplasmic inclusions (shown in Fig. 7, 8), multinucleations in bronchial cells and macrophages, or multinucleated giant cells (shown in Fig. 9, 10). All these findings are summarized in Table 2.

Discussion

The BAL is a useful and safe procedure for the sampling of cellular elements of the lung. As a diagnostic tool, it can be used accurately in various infections, and culture and antibiogram material can also be obtained. This can be very useful in the diagnosis of infections with a sensitivity of 98% and is similar to the bronchial biopsy in sensitivity and specificity [17–19].

The respiratory tract epithelium responds to injury with reactive changes including hyperplasia, metaplasia, degeneration, repair, or inflammation. Although these are often nonspecific changes, others have characteristic cytological features that point to a specific etiology. Furthermore, the epithelium in the distal regions, which comprise the alveoli and smaller branches of the bronchial tree, reacts differently from the epithelium that lines the main bronchi. Sampling these distal areas through the BAL represents a great advantage of this diagnostic method.

Viral pneumonias are frequently associated with prominent hyperplasia of the bronchial and alveolar epithelium, as we have been able to verify in 4 cases studied, in addition to reserve cell hyperplasia. The

Table 2. Cytopathological findings and differential count, determined in studied samples of BAL

Patient	Sample	Nonspecific reactive changes	Virus-specific cytopathic findings	Background	Findings suggestive of bleeding or thrombi	Differential leukocyte count/100 cells
1	1	Absent	Loss of nuclear chromatin pattern, nuclear and cytoplasmic inclusions, multinucleations in bronchial cells and macrophages, multinucleated giant cells	Inflammatory, predominantly histiocytic	Hemosiderin-laden macrophages	10% neutrophils, 5% lymphocytes 0% eosinophils, 85% histiocytes
2	2	Bronchial, alveolar, and reserve cell hyperplasia	Multinucleations in macrophages, lymphocytes with atypical nuclei	Inflammatory, lymphohistiocytic	Absent	10% neutrophils, 30% lymphocytes, 0% eosinophils 60% histiocytes
3	3	Absent	Multinucleations in bronchial cells and macrophages, multinucleated giant cells	Inflammatory and necrotic	Absent	50% neutrophils, 8% lymphocytes, 2% eosinophils 40% histiocytes
4	4	Absent	Absent	Inflammatory, predominantly histiocytic	Absent	2% neutrophils, 3% lymphocytes, 0% eosinophils 95% histiocytes
5	5	Absent	Loss of nuclear chromatin pattern, macrophages multinucleated, atypical lymphocytes, multinucleated giant cells	Inflammatory, predominantly histiocytic	Hemosiderin-laden macrophages	10% neutrophils, 5% lymphocytes, 0% eosinophils 85% histiocytes
6	6	Bronchial cell hyperplasia	Macrophages multinucleated and nuclear inclusions, multinucleated giant cells	Inflammatory, predominantly histiocytic	Absent	5% neutrophils, 4% lymphocytes, 1% eosinophils 90% histiocytes
7	7A	Absent	Absent	Inflammatory, predominantly histiocytic	Hemosiderin-laden macrophages	5% neutrophils, 10% lymphocytes, 5% eosinophils 80% histiocytes
	7B	Absent	Absent	Inflammatory, lymphohistiocytic and necrotic	Hemosiderin-laden macrophages and squamous metaplasia	2% neutrophils, 20% lymphocytes 8% eosinophils 70% histiocytes
8	8	Absent	Macrophages multinucleated, multinucleated giant cells	Inflammatory, predominantly histiocytic	Hemosiderin-laden macrophages	3% neutrophils, 7% lymphocytes, 0% eosinophils 90% histiocytes
9	9	Bronchial, alveolar, and reserve cell hyperplasia	Vacuolar inclusion bodies, macrophage cytoplasmic, multinucleated giant cells	Inflammatory, lymphohistiocytic	Hemosiderin-laden macrophages	15% neutrophils, 15% lymphocytes, 0% eosinophils 70% histiocytes
10	10	Absent	Multinucleations in macrophages	Inflammatory, lymphohistiocytic	Hemosiderin-laden macrophages	25% neutrophils, 30% lymphocytes, 0% eosinophils 45% histiocytes
11	11	Absent	Macrophages multinucleated, multinucleated giant cells	Inflammatory, predominantly histiocytic	Hemosiderin-laden macrophages	3% neutrophils, 4% lymphocytes, 3% eosinophils 90% histiocytes

Table 2 (continued)

Patient	Sample	Nonspecific reactive changes	Virus-specific cytopathic findings	Background	Findings suggestive of bleeding or thrombi	Differential leukocyte count/100 cells
12	12	Absent	Multinucleations in macrophages and lymphocytes with atypical nuclei	Inflammatory, predominantly histiocytic	Hemosiderin-laden macrophages	10% neutrophils, 5% lymphocytes, 0% eosinophils 85% histiocytes
13	13	Absent	Intranuclear and cytoplasmic inclusion bodies in macrophages	Inflammatory, predominantly neutrophilic	Absent	89% neutrophils, 1% lymphocytes, 0% eosinophils 10% histiocytes
14	14	Absent	Loss of nuclear chromatin pattern, multinucleations in bronchial cells and macrophages	Inflammatory, lymphohistiocytic and necrotic	Hemosiderin-laden macrophages	5% neutrophils, 31% lymphocytes, 0% eosinophils 64% histiocytes
15	15	Absent	Loss of nuclear chromatin pattern, multinucleations in bronchial cells and macrophages, macrophage nuclear inclusions	Inflammatory and necrotic	Hemosiderin-laden macrophages	50% neutrophils, 5% lymphocytes, 0% eosinophils 45% histiocytes
16	16	Bronchial and alveolar cell hyperplasia	Loss of nuclear chromatin pattern, multinucleations in bronchial cells and atypical lymphocytes, macrophage cytoplasmic and nuclear inclusions, multinucleated giant cells	Inflammatory, predominantly histiocytic	Hemosiderin-laden macrophages	2% neutrophils, 4% lymphocytes, 0% eosinophils 96% histiocytes

presence of necrotic remains is a frequent finding, especially in those infections caused by *influenza* and *parainfluenza* viruses [20], an aspect also identified in four cases in this study.

Lung injury due to COVID-19 has been described in 263 cases from 28 studies [21]. Findings in biopsy samples included squamous metaplasia [22–28], reactive hyperplasia of pneumocytes [23–37], multinucleated giant cells [24, 26, 29, 30, 32, 38–40], and alveolar hemorrhage with detection of abundant hemosiderin-laden macrophages [23, 24, 26, 27, 29, 35]. Inflammation was also described acute and chronic alveolar space [28]. All of these changes were isolated or associated with diffuse alveolar damage. These changes are positively correlated with the cytopathological findings identified in the BAL samples analyzed in our study.

The integration of the morphological study together with the predominant cell count in the samples can lead to a final pathobiological understanding of the infection. Based on the published literature, BAL samples from healthy individuals contain mainly macrophages (80–90%), few lymphocytes (5–15%), neutrophils (3%), and eosinophils (<1%) [41, 42]. The ratio observed in patients with COVID-19 between neutrophils, lymphocytes, and macrophages is similar to that of other interstitial lung diseases, in which pronounced neutrophilia in BAL samples is generally associated with acute respiratory distress syndrome (ARDS) and acute interstitial pneumonia [43, 44].

In 12/17 of the samples that we studied, a neutrophil count greater than 3% was observed, in the absence of infection or bacterial colonization. Previous studies have also described subsets of patients with increased neutrophils in the absence of concurrent or overlapping bacterial pulmonary infections [28]. However, an increased neutrophil count should prompt the evaluation of a likely concomitant infection. The role of neutrophils in disease progression is evidenced by the maintenance of the host's innate immune defense by these cellular elements, which can initiate and propagate inflammation and thrombosis [45, 46].

Another striking finding was the frequent detection of multinucleated giant cells, whose histological counterpart has recently been well described, in approximately 50% of autopsy studies, which are frequently associated with the late proliferative or fibrotic stage of diffuse alveolar damage (DAD) [25, 29]. Some authors have even proposed that the identification of these cells

could represent a cytopathic effect induced by the virus, describing at the same time the nuclear clearance of pneumocytes as a possible direct effect, calling these covocytes [47, 48].

These viral cytopathic changes were observed in pneumocytes and even viral inclusions were reported [27, 35, 40, 49–52]. Immunohistochemistry has shown positivity against the 2019-nCoV antigen in the alveolar epithelium, desquamated pneumocytes, and macrophages [30] and RNA particles have also been seen in the cytoplasm of numerous multinucleated giant cells [28].

Similarly, in situ hybridization, viral RNA was identified in the tracheal epithelium [28] and some studies have shown SARS-CoV-2 virions by transmission electron microscopy within alveolar macrophage phagosomes [52]. These previous findings also correlate with the cytopathic changes identified by our study in bronchial cells and mainly in macrophages.

The airway epithelium contains a specialized subset of macrophages known as alveolar macrophages, which are a specific type of tissue-resident macrophages that originate in the yolk sac during early embryogenesis. These cells have the capacity to self-heal [53, 54]. However, it has also been shown that bone marrow-derived monocytes are recruited into the lung to repopulate this organ after specific conditions of infection [55].

These macrophages are likely to be an important determinant of early responses to respiratory virus infections due to their abundance and their physical location in the lung. Therefore, they would probably be the first type of immune cells faced by respiratory viruses [56]. In line with this, they are considered the main producers of interferons during infection by respiratory viruses, such as *influenza* virus [55–59] and *respiratory syncytial virus* [59].

In addition, it is considered that the SARS-CoV-2-induced macrophage activation syndrome could be lethal and it is very likely that the heterogeneity of macrophages is involved in determining the severity, so that the variable responses and diverse changes detected in these cellular components could even be important in determining the prognosis of these patients. Similarly, future lines of research could be based on the comparison of such cellular changes according to severity parameters such as age or various baseline conditions [60].

In this cytopathological scenario, the lymphocytes could show cytological abnormalities as well, as observed in peripheral blood [13]. These atypical morphological aspects generally range from plasmacytoid morphology with eccentric nuclei and intracytoplasmic halos [2], to large nuclei and evident nucleoli reminiscent of immunoblasts. In four samples analyzed in our study, small/intermediate sized lymphocytes with convoluted/cerebriform nuclei were observed, with an increased nucleus/cytoplasm ratio.

Morphologic findings of atypical lymphocytes in BAL fluid were also reported [13–15]. The presence of atypical lymphocytes probably reflects the active response of T cells to *SARS-CoV-2* infection, and it should be noted that a study in peripheral blood samples showed that the presence of atypical lymphocytes showed a positive correlation with a better prognosis [61].

In conclusion, BAL is a procedure aimed at obtaining cells from the respiratory tract that can provide valuable and rapid information on the status of infections and interstitial lung diseases. This study has limitations since the observations were made on a sample of limited size, given the high risk of contagion of health workers and laboratory personnel in the collection period corresponding to the first wave. However, it is important to collect and describe as many cytopathological findings as possible, which can provide relevant information for future studies related to the different clinical courses, to finally verify how these pieces fit into the puzzle that represents the study of COVID-19 disease.

Acknowledgments

We thank all patients, their families, and colleagues who with their care and research have worked together during a pandemic with global consequences.

Statement of Ethics

This study protocol was reviewed and approved by the Committee of University Hospital Fundación Alcorcón. The written informed consent was obtained from participants of this study.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Silvio Antonio Galeano Reyes and Patricia Dhimes Tejeda: cytological and statistical analysis and writing of the article. Bárbara Steen: performing the bronchoalveolar lavage technique. Hansely Keret Arcos Orozco and Paloma Ramos Pontón: statistical analysis and writing of the article.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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