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Evaluation of respiratory anatomical-functional sequelae in patients who recovered from COVID-19

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Abstract
Introduction: Coronavirus disease 19 (COVID-19) has been a global public health emergency, with 209.89 million cases of infection with SARS-CoV-2 recorded, resulting in 4,401,675 deaths. After recuperation, it is probable that COVID-19 patients have sequelae of the disease. This study aimed to evaluate the respiratory anatomical–functional sequelae in Mexican patients who recovered from COVID-19.
Methodology: This study included twenty-four patients who recovered from COVID-19 and eight non-infected patients (controls). Participants were screened for SARS-CoV-2 and the presence of IgM/IgG antibodies. Pulmonary function and lung anatomical abnormalities were evaluated by spirometry and computerized tomography.
Results: A total of 45.8% of the patients had pulmonary function with obstructive patterns: 70.8% of recovered cases had COVID-19 Reporting and Data System (CO-RADS) 1, 20.8% CO-RADS 3 and 16.7% CO-RADS 4. A total of 35.3% of patients with CO-RADS 1 also showed bilateral nodal growth; 70.8% of patients tested positive for IgG and 8.4% for IgG/IgM, and 20.8% tested negative for both antibodies.
Conclusions: There were respiratory anatomical and functional sequelae in Mexican patients who recovered from COVID-19, with a high occurrence of pulmonary obstructive patterns in the study population. These observations indicate the importance of the routine evaluation of sequelae in Mexican patients who recovered from COVID-19 and the need for strict follow-up to improve the quality of life of these patients.

Key words: pulmonary sequelae; SARS-CoV-2; COVID-19; coronavirus; computerized tomography scan.

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Introduction
The recent pandemic caused by coronavirus 2 of severe acute respiratory syndrome (SARS-CoV-2), the causal agent of coronavirus disease 19 (COVID-19) [1], has had a global impact due to negative outcomes associated with severe respiratory compromise as a result of pneumonia or acute respiratory distress syndrome (ARDS) [1], coagulation dysfunction [2] and sepsis [3,4]. Since its appearance on December 2019, more than 209 million people worldwide have been infected with SARS-CoV-2 and 4,401,675 patients have died because of COVID-19 [5].

Cell infection associated with SARS-CoV-2 involves the participation of cell surface receptors such as angiotensin-converting enzyme 2 (ACE2) receptor [6] and transmembrane protease serine 2 (TMPRSS2), which cleaves ACE2 and activates the viral protein spike (S) [7-9]. The complex pathophysiology of COVID-19 may be explained in part because these membrane receptors are present in cells of several tissues in the body in addition to those in the respiratory system [10]. Once the virus infects the host cell and is detected, the immunological response gradually triggered against SARS-CoV-2 includes hyperactivation of T cells (with an increase in Th-17 and Th-1) [11] and the activation of alveolar macrophages [12]; the humoral immune response of B cells is necessary for the clearance of cytopathic viruses. Immune cell response triggers a continuous release of proinflammatory cytokines (IL-1β, IL-6, etc.) and tumor necrosis factor alpha (TNF-α), in a process known as a "cytokine storm" [4,13]. At the same time, other molecules (such as GM-CSF, IFNγ, IP-10, MCP-1 and MIP-1α) modulate the chemotaxis and activation of monocytes, macrophages and neutrophils [13]. These processes, combined with the high cytotoxicity of CD8 T cells, may explain the diffuse thickening of the alveolar wall with mononuclear and macrophage infiltration of the spaces and the edema that fills alveolar spaces with consequent formation of hyaline membranes [8,12]. Moreover, the damage to type II pneumocytes, one of the most important targets of SARS-CoV-2, causes a decrease in the production of surfactant, causing atelectasis, which affects lung compliance and worsens hypoxemia [14]. Another aspect related to hypoxemia is the pro-coagulant stimulus due to endothelitis caused by viral infection [15]. Overall, the alveolar-capillary membrane dysfunction that causes alteration of oxygen and endothelial dysfunction results in the formation of microthrombi and affects various systems, causing the complications of COVID-19 related to fatal outcomes [8]. Since COVID-19 involves serious alterations in most of the tissues of the body, many studies have focused on the epidemiological and clinical characteristics of patients with COVID-19 [16]. To date, there are few reports about clinical follow-up of discharged/recovered patients [17]. Some studies suggest that, after discharge, COVID-19 survivors who developed the severe or mild form of the disease may have lung fibrosis [18], neurological [19] and/or bone sequelae associated with ARDS [20]. However,

Table 1. General characteristics of the study population.

Characteristics	Cases (n = 24)	Controls (n = 8)	*p*-value
Sex			
Male n (%)	14 (58.3)	3 (37.5)	0.423
Female n (%)	10 (41.7)	5 (62.5)	
Age (years)	38 ± 10.7	29 ± 10.0	0.020*
Thorax perimeter (cm)	97.9 ± 11.3	-	
Systolic blood pressure (mmHg)	119.3 ± 13.6	116.9 ± 7	0.636
Diastolic blood pressure (mmHg)	78.4 ± 9.4	77.5 ± 4.6	0.8
Heart rate (beats per minute)	75.8 ± 11.4	-	
Breathing frequency	17.1 ± 2.1	-	
Temperature (°C)	36.2 ± 0.3	36.1 ± 0.5	0.67
Blood oxygen level (%)	93.8 ± 1.7	94.7 ± 2.7	0.351
Risk factors n (%)			
Hypertension	2 (8.3)	-	-
Arrhythmias	1 (4.2)	-	-
Peripheral venous insufficiency	4 (16.7)	-	-
Diabetes mellitus type 2	1 (4.2)	-	-
Gestational diabetes	-	-	-
Tobacco consumption	4 (16.7)	3 (37.5)	0.396
Alcohol consumption	15 (62.5)	3 (37.5)	
Drugs consumption	1 (4.2)	1 (12.5)	
Exposure biomass smoke	2 (8.3)	1 (12.5)	
Neoplasia	2 (8.3)	-	-
Rheumatoid arthritis	1 (4.2)	-	-
Allergic rhinitis	2 (8.3)	-	-
Fatty liver	1 (4.2)	-	-
Nutritional condition n (%)			
Normal	5 (20.8)	5 (62.5)	0.199
Overweight	11 (45.8)	2 (25.0)	
Obesity	7 (29.2)	1 (12.5)	
Mucosa color n (%)			
Normal	19 (79.2)	-	-
Pale	2 (8.3)	-	-
Spirometry n (%)			
Normal	11 (45.8)	8 (53.3)	0.033*
Restrictive	1 (4.2)	0	
Obstructive	11 (45.8)	0	
Treatment n (%)			
Non-pharmacological	11 (45.8)	0	-
Pharmacological	14 (58.3)	0	

*Significant difference.

pulmonary function and lung anatomy after clinical recovery and SARS-CoV-2 clearance has not been studied in detail. The aim of this study was to evaluate the respiratory anatomical–functional sequelae in Mexican patients who recovered from COVID-19. Antibody production one month after SARS-CoV-2 clearance was also tested.

Methodology

Patients

This study was approved by the Ethics and Research Committee of the Academic Unit of Human Medicine and Health Sciences of the Universidad Autonoma de Zacatecas "Francisco García Salinas" (ID: UAMHYCS 001/2020) and by the Alpha Medical Center Research Committee (ID: AMCCI-FSARSC2-006/-007). The study was thereafter announced live via social media in the presence of representatives of the main local communication media. People over 18 years old who lived in the state of Zacatecas, Mexico, with a previous diagnosis of COVID-19 by molecular test and with one month elapsed since their viral clearance, were eligible to participate; they were included in the study as COVID-19 recovered cases. Additionally, eight healthy control subjects were also included (Table 1). The patients were recruited during June 2020 in the Clínica Universitaria, a primary health center of the Academic Unit of Human Medicine and Health Sciences. Patients were informed of the potential but unproven benefits and harm associated with their participation in the study. There were no exclusion criteria for this study. From each participant who granted their informed consent, a questionnaire regarding demographic and clinical data, signs and symptoms related to COVID-19 and data of their pharmacological therapy during COVID-19 was distributed.

Clinical procedures

Spirometry: After physical examination and anthropometric measurements, each participant underwent baseline spirometry and a control spirometry post-bronchodilator (100 µg of inhaled salbutamol). Each patient was clearly instructed regarding the appropriate manner to carry out the procedure, in which a pneumologist showed the patient how to perform at least three exhalations of 6 seconds or more in each execution. In this study, a decrease in forced vital capacity (FVC) less than 80% of that predicted was considered as the reference value for a restrictive pattern. An obstructive pattern was diagnosed when a decrease in the forced expiratory volume (FEV1)/FVC ratio was less than 70% of that predicted, and/or when there was an improvement in FEV1 of 12% or an increase of more than 200 ml in FEV1 in spirometry 15 minutes after administering the bronchodilator [21,22]. Spirometry was carried out with a Cardinal Health Spiro spirometer (Cardinal Health, Ltd, Rochester Kent, UK), and data were collected and processed using the CareFusion Spirometry PC software v.1.05 (CareFusion Corporation, San Diego, CA, USA).

Computerized tomography: Each COVID-19 recovered participant was examined for the identification of lung anatomical abnormalities using computerized tomography (CT). Images obtained by CT were examined for both lungs. Chest CT images were evaluated for the presence of incidental abnormalities and those related to COVID-19, such as sub-pleural ground-glass opacities and consolidation, mixed ground-glass opacities and consolidation, nodules, septal thickening, reticulation, architectural distortion, sub-pleural bands, perilobular opacities, traction bronchiectasis and bronchial wall thickening. The COVID-19 Reporting and Data System (CO-RADS) was used to provide a standardized assessment scheme that simplifies reporting with a five-point scale (1–5) of suspicion for pulmonary involvement of COVID-19 in chest CT. Experienced radiologists reviewed all chest CT images independently.

SARS-CoV-2 diagnosis and antibody measurements

A total of 32 pharyngeal and 32 nasopharyngeal exudates collected with swabs in 2.5 mL of viral transport medium were obtained from patients and transported in a triple-walled container at 4 °C to the Molecular Medicine Laboratory of Universidad Autónoma de Zacatecas. This laboratory is a COVID-19 diagnosis laboratory authorized by the Instituto de Diagnóstico y Referencia Epidemiológicos "Dr. Manuel Martínez Báez", which is the institution of the Ministry of Health of Mexico in charge of diagnosis, control, referral, research and technological development for the surveillance of epidemiological diseases. Exudate samples were screened for SARS-CoV-2 using the Centers for Disease Control and Prevention (CDC) real-time RT-PCR Panel (IDT—Integrated DNA Technologies, IA, USA) and interpreted according to the manufacturer's instructions. The IgM and IgG antibodies against SARS-CoV-2 were evaluated in total blood using the COVID-19 IgG/IgM Rapid Test Cassette (Premier Biotech, MN, USA).

Statistical analysis

General characteristics of the study population were represented as mean ± SD and percentages. Comparisons of the risk factors and the clinical findings among the groups were performed using chi-square or Fisher's exact test for categorical variables and t-test or Mann–Whitney U-test for continuous variables. Statistical analysis was carried out with the IBM-SPSS Statistics for Windows v21.0 (IBM Corp., Armonk, NY, USA) software and considering a significance level of 0.05.

Results

Twenty-four patients reported as recovered from COVID-19 were included in the study. Table 1 shows the general characteristics of the participants in the study. Fourteen cases were male and 10 were female, with a mean age of 38 years (±10.8). A total of 62% of the patients indicated consumption of alcohol and 16.6% reported using tobacco. The most frequent comorbidity was peripheral venous insufficiency, which was present in 16.6% of the patients. A total of 45% of the recovered cases were overweight, and 29.2% had obesity.

Table 2. Signs and symptoms of the recovered COVID-19 participants during and after disease.

Signs and symptoms	COVID-19 cases (n = 24) During COVID-19 n (%)	One month after virus clearance n (%)
Fever	18 (75)	-
Cough	13 (54.1)	3 (12.5)
Headache	20 (83.3)	1 (4.2)
Dyspnea	16 (66.6)	3 (12.5)
Irritability	11 (45.8)	-
Diarrhea	10 (41.7)	-
Chest pain	15 (62.5)	1 (4.2)
Chills	16 (66.6)	-
Odynophagia	15 (62.5)	3 (12.5)
Myalgia	17 (70.8)	1 (4.2)
Arthralgia	13 (54.1)	-
General health status	21 (87.5)	-
Rhinorrhea	10 (41.7)	1 (4.2)
Polypnea	12 (50%)	-
Vomiting	12.5	-
Abdominal pain	5 (20.8)	-
Conjunctivitis	11 (45.8)	-
Cyanosis	2 (8.3)	-
Anosmia	16 (66.6)	7 (29.2)
Dysgeusia	15 (62.5)	5 (20.9)
Skin manifestations	5 (20.8)	1 (4.2)
Others		
Apnea	1 (4.2)	1 (4.2)
Polyuria	1 (4.2)	-
Retroocular pain	1 (4.2)	1 (4.2)
Weight loss	2 (8.3)	2 (8.3)
Hemoptysis	1 (4.2)	-
Asthenia	-	1 (4.2)
Fasciculations	-	2 (8.3)

Table 2 summarizes the signs and symptoms of the patients studied, classified as during and after COVID-19. The most frequent signs and symptoms at COVID-19 diagnosis were general health status in 21 (87.5%) participants, headache in 20 (83.3%), fever in 18 (75%) and myalgia in 17 (70.8%). During COVID-19, five of the participants (20.8%) needed hospitalization and three (8.3%) required mechanical ventilator support. All (100%) of the participants received pharmacological therapy, with the use of antipyretics (95.8%), antivirals (12.5%), hydroxychloroquine (8.3%) and tocilizumab (4.2%). A total of 40% of the participants claimed to have been vaccinated against influenza A/H1N1 in the last year. After COVID-19, the most persistent symptoms were anosmia (29.2%) and dysgeusia (20.9%). Asthenia and fasciculation were manifested after COVID-19 in 4.2 and 8.3% of the participants, respectively.

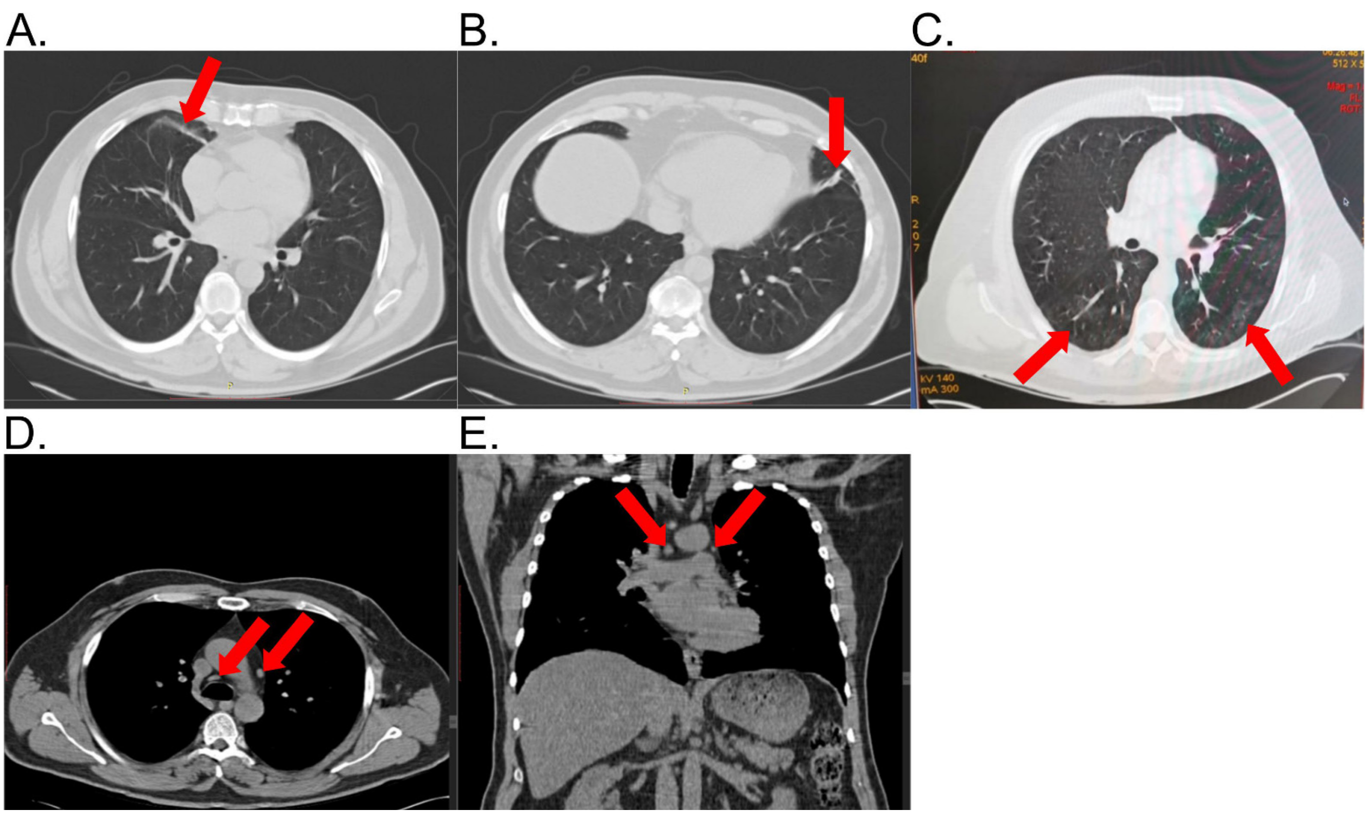
To evaluate lung function, each patient underwent spirometry which involved three exhalations of 6 seconds or more in each execution before and after a bronchodilator was used (see Materials and Methods section for details). The obtained patterns regarding lung function of COVID-19 recovered participants were compared with those obtained for the healthy participant control (Table 1). Eleven patients (45.8%) had an obstructive pattern, and one patient (4.2%) showed a restrictive pattern. In patients with obstructive patterns, the mean FEV1, FVC, and FEV1/FVC ratio was 3.4 ± 0.42 L, 3.5 ± 0.75 L, and 74.3%, respectively. In patients with normal lung function, mean FEV1, FVC and FEV1/FVC was 3.7 ± 0.32 L, 3.7 ± 0.55 L and 80.3%, respectively ($p < 0.05$). The values of FEV1, FVC and FEV1/FVC for patients with restrictive pattern were 2.9 L, 3.3 L and 70.3%, respectively. The results of one patient were excluded because he had steroid treatment at the moment of testing.

Table 3 and Figure 1 summarize the anatomical lung findings and show the representative images obtained by CT for the participants. Considering the CT of the chest with a pulmonary window, 17 patients (70.8%) had a normal scan with CO-RADS 1 score, five patients (20.8%) had CO-RADS 3 and two patients (8.3%) had CO-RADS 4. Atelectasis was observed in two (8.3%) patients. The most frequent atypical finding was lymphadenopathy in eight (33.3%) patients with CO-RADS 1. Other incidental findings were thyroid nodules in two patients and residual pneumothorax and hiatal hernia in one patient.

Table 3. Summary of symptoms and clinical and immunological findings observed in the COVID-19 recovered participants.

ID	Hospitalization	Spirometry pattern	CO-RADS/other CT finding	Rapid test	
				IgM	IgG
1	No	Obstructive	CO-RADS 1	-	-
2	No	Normal	CO-RADS 1	-	+
10	No	Normal	CO-RADS 1	-	+
11	No	Obstructive	CO-RADS 1	-	-
28	No	Obstructive	CO-RADS 1	-	+
29	Yes	Normal	CO-RADS 1	-	-
30	Yes	Obstructive	CO-RADS 1	-	+
31	No	Normal	CO-RADS 1	-	+
32	No	Obstructive	CO-RADS 1	-	+
39	No	Obstructive	CO-RADS 1	-	+
41	No	Normal	CO-RADS 1	-	-
6	No	Normal	CO-RADS 1/Lymph node growth	-	-
7	No	Obstructive	CO-RADS 1/Lymph node growth	-	+
8	No	Obstructive	CO-RADS 1/Lymph node growth	-	+
35	Yes	Restrictive	CO-RADS 1/Lymph node growth	-	+
36	No	Obstructive	CO-RADS 1/Lymph node growth	-	+
37	No	Normal	CO-RADS 1/Lymph node growth	-	+
3	No	Normal	CO-RADS 3	+	+
4	No	Obstructive	CO-RADS 3	-	+
9	No	Not valuable	CO-RADS 3	-	+
38	No	Obstructive	CO-RADS 3	-	+
40	No	Normal	CO-RADS 3	-	+
5	Yes	Normal	CO-RADS 4	-	+
34	Yes	Normal	CO-RADS 4	+	+

Figure 1. Computerized tomography findings of COVID-19 recovered patients.



Each participant was screened for lung anatomical abnormalities using computerized tomography (CT). The figure shows CT of the chest with pulmonary window for A. a male patient, 47 years old, with lineal right atelectasis (row) and B. lineal left atelectasis (row); C. a male patient, 48 years old with peripheral and bilateral ground-glass opacities; D. and E. a male patient, 26 years old, with the presence of abnormal lymph node growths (4 and 6 level).

The average time elapsed since SARS-CoV-2 clearance was 56 days in the study population. After this time, the result of SARS-CoV-2 screening by RT-PCR showed positive results for one patient with a Cq value of 34. In addition, the rapid test for IgM/IgG showed positive results for the presence of IgG in 19 (79.1%) patients and of IgM in two patients (8.3%); negative results were found in five (20.8%) COVID-19 recovered cases (Table 3).

Discussion

Depending on its severity, COVID-19 involves important alterations in most tissues of the body, with variable severity ranging from an asymptomatic manifestation to a fatal outcome [17]. After recuperation, it is highly probable that patients who suffered COVID-19 may present sequelae of the disease [17-20]. Therefore, this study aimed to evaluate the respiratory anatomical–functional sequelae in Mexican patients who recovered from COVID-19. Since no studies have evaluated the lung both functionally and anatomically following SARS-CoV-2 clearance, we extrapolated the information in functional terms from previous epidemics caused by viruses such as influenza A/H1N1 and/or SARS and compared it with our findings, as described in the following paragraphs.

Comparing the alterations in respiratory mechanics with those of other respiratory viral infections is dependent on the stage in which the disease is found [23]. However, in infections limited to the upper airways, where the imaging findings are normal, they present with physiological alterations of variable duration (sub-clinical). This suggests the involvement of the lower airways because their participation is usually asymptomatic, but it is the main anatomic site of involvement in uncomplicated viral infection. If the viral infection progresses to acute lung injury or ARDS, gas exchange and tissue transport of oxygen are compromised because of hypoventilation, alterations in diffusion, short circuits and alterations of ventilation-perfusion. These facts reflect the pathological abnormalities of patients previously described in the SARS epidemic and other pandemics caused by the influenza virus: diffuse alveolar damage, necrotizing bronchiolitis and alveolar damage with alveolar hemorrhage [23]. In pneumonia of viral origin, it has been shown that approximately 50% of patients initially present with a restrictive functional pattern with decreased FVC <80% of that predicted. The remaining 50% show an obstructive pattern (decrease in FEV1/FVC ratio); both changes are reversible even after 2 months [23]. Of our 24 recruited patients, 19 (79%) had an infection that did not require hospitalization (only symptomatic home treatment) and when recruited they had a radiological classification of CO-RADS 1. Only 5 (21%) of the studied patients required hospitalization due to CURB-65 classification of 2 points (at time of writing: CO-RADS 1, 3 and 4). However, the obstructive pattern was found to be persistent in 11 (45.8%) patients and only one patient (4.2%) showed a restrictive pattern. This proportion was dissimilar to that observed in the SARS epidemic of influenza A/H1N1, in which the influence of smoking and other obstructive diseases, such as chronic obstructive pulmonary disease (COPD) and asthma, were not present as possible biases in the findings. In their chest CT scan, patients with an obstructive pattern correlated with a CO-RADS 1 radiological classification, with no evidence of injury because, as previously discussed, the viral condition affects the small airways. It is important to note that an atypical finding identified in this protocol was lymphadenopathy in six (25%) of our patients; persistent atelectasis in two other patients was also found. Interestingly, the rapid test showed the presence of IgG in all patients with lymph node growth, indicating that the lymph nodes were still active. With the information of long-term changes in lung function in previous pandemics, such as influenza A/H1N1 and SARS, it appears that the respiratory function tests and anatomical findings from CT normalize for most patients. However, there may also be anatomical–functional sequelae when the infection has been severe [18]. Different degrees of fibrosis or slow recoveries seen in some of the cases are possibly due to an autoinflammatory reaction caused by pyroptosis in its pathogenesis [18,24], which causes cells to burst, thereby releasing hidden antigens that can undergo a transition to the immune system, triggering the inflammatory process [24]. These facts indicate the need to maintain long-term research on respiratory function in subjects who were infected with SARS-CoV-2.

Finally, as previously reported, in the brain the olfactory bulb may be infected by SARS-CoV-2, which leads to an inability to smell and might trigger other neurodegenerative diseases in COVID-19 recovered patients. Therefore, the observed persistence of symptoms in a portion of our patients, such as anosmia (29.2%) and dysgeusia (20.9%), is an important finding that could be attributed to the slow recovery of the involved nerve pathways. However, additional studies are necessary to evaluate their future significance in patient health if this may trigger other consequences related to COVID-19.

Conclusions

Our results reflect a high prevalence of respiratory anatomical and functional sequelae in Mexican patients who recovered from COVID-19, with a high occurrence of pulmonary obstructive patterns in the study population. Lymphadenopathy was one of the most important atypical CT findings, present in 33.3% of our patients; this finding was linked with the presence of IgG antibodies, reflecting the activity of the lymph nodes. These observations indicate the importance of the routine evaluation of the sequelae in Mexican patients who recovered from COVID-19 and the need for strict follow-up to ensure therapies are appropriate for improving their quality of life.

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Authors' Contributions

MLMF conceived and designed the study and analysis. MLMF and JIBA coordinated patient recruitment. JRMT, GACP, IGV, EGV, LSHM and LAHM recruited patients and collected the clinical data. IGV, AJM and JRGC performed laboratory tests. JRMT, EGV, OGMZ, CCV, ADTG and AJM contributed data and/or analysis tools. JRMT, GACP, IDE and IPRS performed the analysis. MLMF and JIBA were the primary authors contributing to the coordination of the manuscript, literature review and writing, editing and overseeing of the paper as a whole, including the submission process. All authors contributed to the article and approved the submitted version.

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