Estudio multidimensional de la condición post COVID-19.

Universidad de Granada

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ÍNDICE DE CONTENIDOS

1. RESUMEN

INTRODUCCIÓN

La enfermedad por coronavirus 2019 (COVID-19) está aún lejos de finalizar. Actualmente se ha adoptado el término de "condición post COVID-19" (PCC) para referirnos a la sintomatología nueva, recurrente o continua, a las secuelas secundarias a los daños orgánicos producidos y, a los efectos de la hospitalización que aparecen después del proceso agudo causado por el coronavirus tipo 2 causante del síndrome Respiratorio Agudo Severo (SARS-CoV-2).

Nuestro objetivo general es investigar de forma multidimensional sobre la CPP, lo cual haremos profundizando sobre: la sintomatología persistente, los daños orgánicos residuales, el papel del PET TC en el seguimiento y, las relaciones entre las distintas variables que pueden predisponer al desarrollo de la PCC.

Esta tesis doctoral es el resultado de 3 trabajos de investigación sobre la CPP realizados por la doctoranda, todos ellos publicados en revistas indexadas en el Journal Citation Report (JCR).

METODOLOGIA.

Los artículos que componen esta tesis son estudios prospectivos de pacientes post COVID que fueron remitidos a las consultas de Neumología, del Hospital Universitario Virgen de las Nieves (HUVN) en Granada, España. En concreto;

Artículo 1: Es un estudio de cohortes de pacientes hospitalizados (o no) por COVID-19, que completaron un seguimiento de 6 meses de evolución.

Artículo 2: Es un estudio de pilotaje sobre una cohorte naturalística de una serie de casos que fueron seguidos 3 meses después del COVID-19.

Artículo 3: Es un estudio de cohortes de pacientes hospitalizados por COVID-19 que completaron un seguimiento de 1 año de evolución.

Todos los pacientes fueron incluidos de forma progresiva en diferentes revisiones médicas y, las variables independientes exploradas en los diferentes trabajos fueron: los factores demográficos, las comorbilidades médicas, la clínica persistente, variables del ingreso hospitalario, de la función pulmonar y, de laboratorio, junto con los hallazgos radiológicos observados en TACAR (TCAR) de tórax y PET TC.

RESULTADOS

Artículo 1: 217 pacientes completaron el seguimiento y, 154 pacientes (73,3%) seguían presentando clínica residual 6 meses después de COVID-19. Encontramos asociación ($p<0,05$) significativa entre el género del paciente con las siguientes variables: fatiga, artralgias, fiebre, disnea, trastornos emocionales, depresión, déficit cognitivo, hemoglobina, bilirrubina total y ferritina. Las enfermedades respiratorias previas (ERP) y el estado de hospitalización están fuertemente asociados con características demográficas específicas, síntomas clínicos, salud mental y pruebas de función respiratoria (PFR) basadas en características únicas y múltiples de PCC.

Artículo 2: A los 2-3 meses del alta, 11 pacientes (55%) seguían mostrando captaciones patológicas, con linfadenopatía mediastínica hipermetabólica en el 90,9%. Eran pacientes de edad avanzada e índice de Charlson elevado; al ingreso presentaban cansancio y disnea junto con recuentos más bajos de hemoglobina y linfocitos. El valor de captación estandarizados máximo (SUV pico) de las lesiones diana y el de glucolisis tumoral total (TLG) pulmonar se correlacionaron significativamente con los reactivos de fase aguda y los recuentos de glóbulos blancos al ingreso, durante la estancia hospitalaria y 2-3 meses después del alta.

Artículo 3: Del total de 157 pacientes que completaron el estudio, 49 (31,2%) cumplían criterios de PFR anormal a los 12 meses. El análisis multivariante de los pacientes con dicho deterioro funcional mostró una asociación significativa ($p<0,05$) con: El índice de Charlson elevado y, las variables al ingreso de radiografía de tórax con neumonía de distribución central y/o mixta, anemia, estancia en unidad de cuidados intensivos (UCI) y, necesidad de tratamiento corticoideo. Así mismo, los valores anormales de alfa 1 antitripsina (AAT) junto con el genotipado alélico deficiente Pi*MZ mostraban esta asociación.

CONCLUSIONES

Artículo 1: Tras 6 meses de la infección aguda por SARS-CoV-2, se verifica la presencia de condición post COVID-19 entre nuestros pacientes. Su desarrollo es independiente de la gravedad de la enfermedad durante la fase aguda y, el tener o no comorbilidades respiratorias previas. No obstante, sí esta influenciado por el género. En concreto, se objetiva como el sexo femenino es un factor favorable para la persistencia de la sintomatología.

Artículo 2: Las pruebas de función pulmonar junto con el PET-TC con $[^{18}F]$FDG son más sensibles que la TAC sola para identificar candidatos con secuelas pulmonares después del COVID-19.

Artículo 3: La presencia de niveles AAT bajos y/o genotipado alélico deficiente Pi*MZ, el índice de Charlson elevado, la presencia al ingreso por COVID-19 de; radiografía de tórax con neumonía, anemia, estancia en UCI y, necesidad de bolos de metilprednisolona pueden predisponer al deterioro de la función pulmonar a largo plazo.

2. ABSTRACT:

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is still far from over. The term "post-COVID condition" (PCC) has now been adopted to refer to new, recurrent or continuous symptomatology, to sequelae secondary to organ damage and to the effects of hospitalization that appear after the acute process caused by the coronavirus type 2 causing Severe Acute Respiratory Syndrome (SARS-CoV-2).

The overall objective is to investigate in a multidimensional way on PPC, which we will do by investigating: persistent symptomatology, residual organ damage, the role of PET CT in the follow-up and the relationships between the different variables that may predispose to the development of PCC.

This doctoral thesis is the result of 3 research works on PPC carried out by the PhD student, all of them published in journals indexed in the Journal Citation Report (JCR).

MATERIAL AND METHODS

The articles that make up this thesis are prospective studies of post COVID patients who were referred to the Pneumology Department of the Hospital Universitario Virgen de las Nieves (HUVN) in Granada, Spain.

Article 1: Prospective cohort study of patients hospitalized (or not) for COVID-19 who completed a 6-month follow-up of evolution.

Article 2: Pilot study on a naturalistic cohort of a series of cases followed up 3 months after COVID-19.

Article 3: Prospective cohort study of patients hospitalized for COVID-19 who completed a follow-up of 1 year of evolution.

RESULTS

Article 1: 217 patients completed follow-up and 154 patients (73.3%) still had residual clinical symptoms 6 months after COVID-19. We found significant association ($p<0.05$) between patient gender with the following variables: fatigue, arthralgias, fever, dyspnea, emotional disturbances, depression, cognitive decline, hemoglobin, total bilirubin and ferritin. Previous respiratory disease (PRD) and hospitalization status are strongly associated with specific demographic characteristics, clinical symptoms, mental health, and respiratory function tests (PFT) based on single and multiple features of PCC.

Article 2: At 2-3 months after discharge, 11 patients (55%) were positive, with hypermetabolic mediastinal lymphadenopathy in 90.9%. They were elderly patients with an

elevated Charlson index; on admission they presented with fatigue and dyspnea along with with lower hemoglobin and lymphocyte counts. Peak standardized uptake value (peak SUV) of the target lesion and pulmonary total tumor glycolysis (TLG) were significantly correlated with acute phase reactants and white blood cell counts on admission, during hospital stay and 2-3 months after discharge.

Article 3: Of the 157 patients who completed the study, 49 (31.2%) met criteria for abnormal PFT at 12 months. Multivariate analysis of patients with such functional deterioration showed a significant association($p<0,05$) with the following variables: elevated Charlson index and, variables upon admission of chest X-ray with the presence of pneumonia of central and/or mixed distribution, anemia, intensive care unit (ICU) stay and need for corticosteroid treatment. Likewise, we found a significant association between such pulmonary impairment and abnormal alpha 1 antitrypsin (AAT) values along with Pi*MZ deficient allelic genotyping.

CONCLUSIONS:

Article 1: After 6 months of acute SARS-CoV-2 infection, the presence of post-COVID-19 condition is verified among our patients. Its development is independent of the severity of the disease during the acute phase and, having or not previous respiratory comorbidities. However, it is influenced by gender. Specifically, female sex is a favorable factor for the persistence of the symptomatology.

Article 2: Respiratory function testing (PFT) in conjunction with [18F] FDG PET-CT is more sensitive than CT alone in identifying candidates with pulmonary sequelae after COVID-19.

Article 3: The presence of low AAT levels and/or Pi*MZ deficient allelic genotyping, high Charlson index, the presence on admission of; chest x-ray with pneumonia, anemia, ICU stay and, need for methylprednisolone boluses contribute to such worsening of pulmonary function in the long term.

3. ABREVIATURAS Y SIGLAS

PCC: *Condición post COVID-19.*

COVID-19: *Enfermedad por coronavirus 19.*

SARS-CoV-2: *Coronavirus de tipo 2 causante del síndrome respiratorio agudo severo.*

SARS-1: *Síndrome respiratorio agudo severo.*

MERS: *Síndrome respiratorio de Oriente Medio.*

JCR: *Journal Citacion Report (Factor de impacto).*

TAS*: Tasa de ataque secundario.*

R0: *Número básico de reproducción.*

Re*: Número reproductivo efectivo.*

IAM*: Infarto agudo de miocardio.*

SCA*: Síndrome coronario agudo.*

ACV: *Accidente cerebrovascular.*

CAD: *Cetoacidosis diabética.*

FDA: *Administración de Alimentos y Medicamentos de los Estados Unidos (EEUU).*

AEMPs: *Agencia Española de Medicamentos y Productos Sanitarios.*

OMS*: Organización mundial de la salud.*

NICE*: Instituto Nacional para la Excelencia en Salud y Atención del Reino Unido.*

CDC*: Centro de control y prevención de enfermedades.*

FR*: Factores de riesgo.*

IMC: *Índice de masa corporal.*

TCAR: *Tomografía computarizada de alta resolución.*

TC: *Tomografía computarizada.*

EPID: *Enfermedad pulmonar intersticial difusa.*

PFR: *Pruebas de función pulmonar.*

FEV1: *Volumen espiratorio forzado en el primer segundo.*

FVC: *Capacidad vital forzada.*

FEV1/CVF: *Relación FEV1/FVC.*

RV: *Volumen residual.*

CRF: *Capacidad funcional residual.*

TLC: *Capacidad pulmonar total.*

DLCO: *Prueba de difusión de monóxido de carbono.*

KCO: *Constante de difusión del monóxido de carbono.*

TM6M: *Test de la marcha de los 6 minutos.*

SERAM: *Sociedad Española de Radiología Médica.*

CI: *Intervalos de confianza.*

OR: *Razones de probabilidades (Odd ratio).*

ERP: *Enfermedad respiratoria preexistente.*

RT-PCR: *Reacción en cadena de la polimerasa con transcripción inversa.*

RADTs: *Test rápidos de antígenos virales.*

Ct: *Umbral de ciclo.*

TEP: *Embolia pulmonar.*

ETEV*: Enfermedad tromboembólica venosa.*

TVP*: Trombosis venosa profunda.*

PCR*: Proteína C reactiva.*

PET-TC*: Tomografía de emisión de positrones.*

18F-FDG: *[18F]- 2- fluorodesoxiglucosa.*

18F-FDG-PET/CT: *18F-fluorodesoxiglucosa- PET/TC.*

MTV*: Volumen metabólico de la lesión.*

HUVN*: Hospital Universitario Virgen de las Nieves.*

ROI*: Regiones de interés.*

DE*: Desviación estándar.*

SUV: *Valores de captación estandarizados.*

SUVbw: *SUV normalizado por peso corporal.*

SUL: *SUV normalizado por masa corporal magra.*

SUVmax*: Volumen de captación estandarizado máximo; Captación/volumen del vóxel de máxima captación.*

TLG*: Glucolisis total de la lesión.*

DM2: *Diabetes Mellitus tipo 2.*

EPOC: *Enfermedad pulmonar obstructiva crónica.*

SDRA: *Síndrome de distrés respiratorio agudo.*

CRS*: Síndrome de liberación de citoquinas.*

NAbs: *Anticuerpos neutralizantes.*

Índice NLR: *Índice neutrófilo-linfocito.*

IL-6*: Interleuquina 6.*

DAAT: *Déficit de alfa 1 antitripsina.*

AAT: *Alfa 1 antitripsina.*

IOT: *Intubación orotraqueal.*

UCI: *Unidad de cuidados intensivos.*

VMNI*: Ventilación mecánica no invasiva.*

VIH: *Virus de la inmunodeficiencia humana.*

mMRC: *Escala modificada de disnea.*

LDH*: Lactato deshidrogenasa.*

AST: *Aspartato transaminasa.*

ALT: *Alanina aminotransferasa.*

NAAT: *Prueba de ampliación de ácidos nucleicos.*

ARN*: Ácido ribonucleico.*

ARNm*: ARN mensajero.*

ADN*: Ácido desoxirribonucleico.*

RBD: *Dominio receptor de unión.*

ACE2: *Enzima convertidora de angiotensina 2.*

AT2: *Angiotensina 1.*

VOC*: Variantes de preocupación.*

VOI: *Variantes de interés.*

PAMPs: *Patrones moleculares asociados a patógenos.*

TLR: *Receptores tipo toll.*

TNFα: *Factor de necrosis tumoral alfa.*

CID*: Coagulación intravascular diseminada.*

DD: *Dímero D.*

TP*: Tiempo de protrombina.*

TTPa*: Tiempo de protrombina parcial activado.*

SIM*: Síndrome inflamatorio multisistémico.*

IHME*: Instituto de Métricas y Evaluación de la Salud.*

CE: *Comisión europea.*

TFGe: *Tasa de filtración glomerular.*

VEB: *Virus Epstein Barr.*

4. INTRODUCCIÓN:

4.1 Breve Historia del SAR-CoV-2.

El 31 de diciembre de 2019, la Comisión municipal de Salud y Sanidad de Wuhan (China) notificó a la Organización Mundial de la Salud (OMS) un grupo de casos de neumonía de origen desconocido, con una exposición colectiva previa a un mercado de pescado y animales vivos en la provincia de Wuhan, China[1]. El 7 de enero de 2020 se identificó la secuenciación del genoma completo indicando que el agente causante era un betacoronavirus del mismo subgénero que el virus del síndrome respiratorio agudo severo (SARS). Por esto, el Grupo de estudio de coronavirus del comité Internacional de taxonomía de Virus propuso que se denominara coronavirus del síndrome respiratorio agudo severo tipo 2 y la enfermedad que producía COVID-19[3]. Fue tal la transmisión viral desde ese momento, que el 11 de marzo la OMS declaró la situación de pandemia mundial[4].

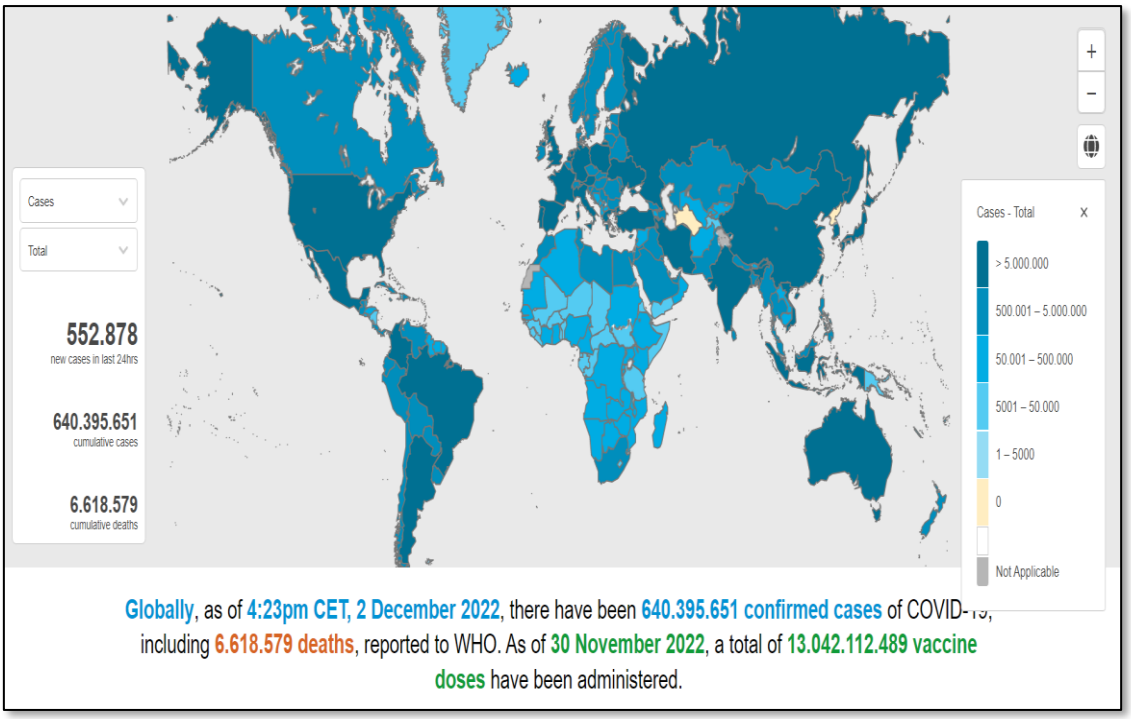

Figura 1. Casos confirmados y muertes debidas al COVID-19 a 4 de diciembre de 2022. Tomada de *WHO Coronavirus (COVID-19) Dashboard | WHO Coronavirus (COVID-19) Dashboard With Vaccination Data*[2].

Los coronavirus son virus que afectan tanto a animales como humanos. Producen infecciones respiratorias agudas sobre todo en época de finales de otoño, invierno e inicio de la primavera, desde leves hasta graves y/o mortales. Se consideran virus endémicos, epidémicos y pandémicos, con epidemias producidas por ellos en las últimas dos décadas[5;

en 2002 causada por el betacoronavirus SARS-CoV(coronavirus del síndrome respiratorio agudo grave) y, en 2021 producida por el MERS-CoV (coronavirus del síndrome de Oriente Medio)[6-7] (Figura 2).

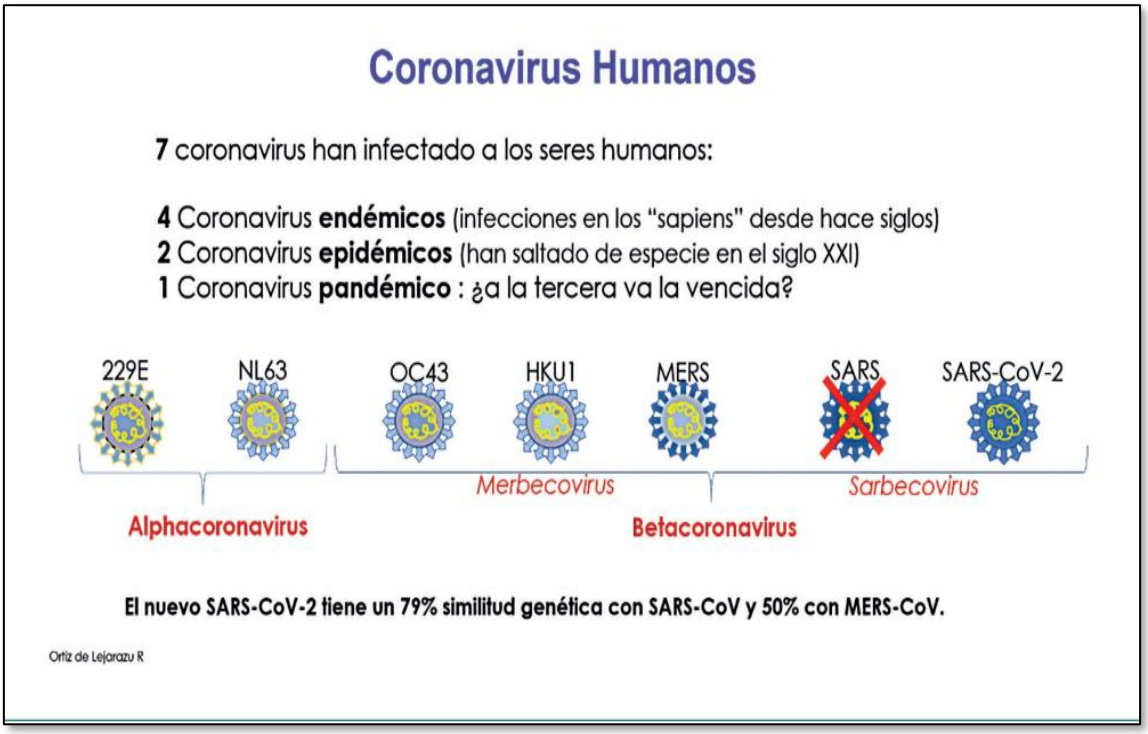

Figura 2. Clasificación epidemiológica de las especias de coronavirus humados. Tomado de Sanz I[5].

4.1.1. Características biológicas principales.

Se clasifican como una familia del orden de los Nidovirales[4], es decir, se replican utilizando un conjunto "anidado" de ARNm, que a su vez es de cadena sencilla y polaridad positiva, siendo su genoma el mayor de la familia de los ribovirus (25-32kb6). Se dividen en cuatro géneros: coronavirus alfa, beta, delta y gamma, siendo los coronavirus humanos (HCoV) el tipo alfa (HCoV-229E y HCoV-NL63) y betacoronavirus (HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV y SARS CoV-2).

Su nombre deriva porque su aspecto por microscopia electrónica es redondeado con espículas sobre una estructura superficial que recuerda a una corona solar[8] (Figura 3).

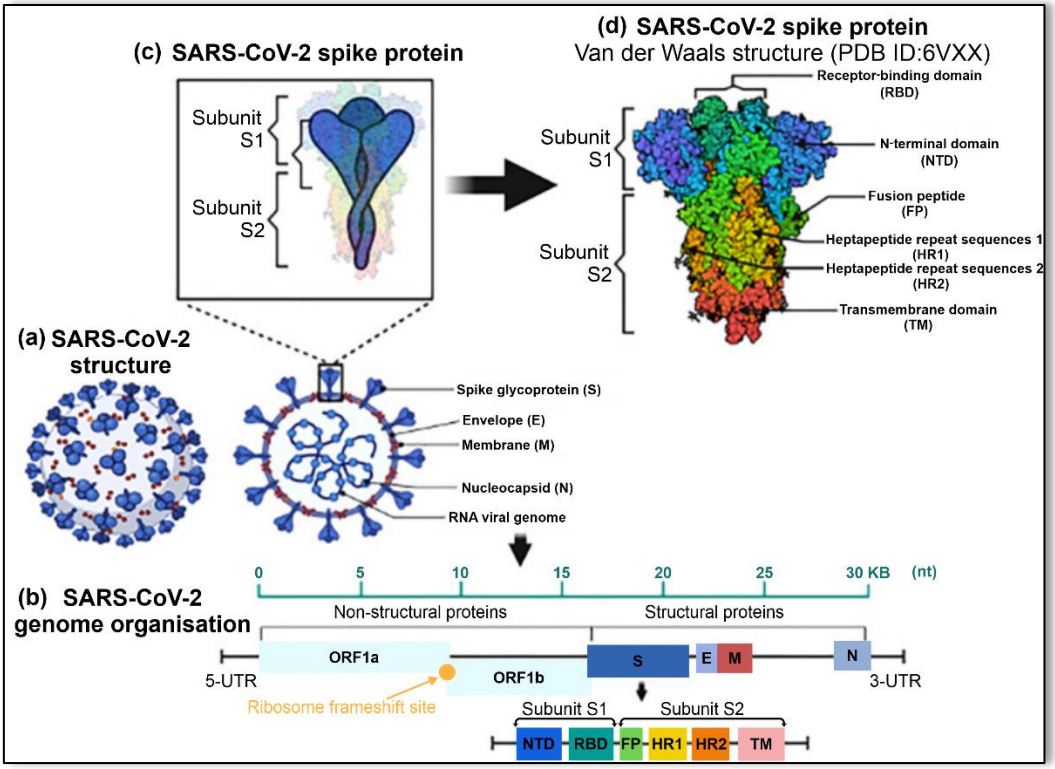

Figura 3. Imagen de la estructura de los coronavirus. Tomado de Hadj I[12].

Su genoma codifica diferentes proteínas estructurales y no estructurales. Las 5 proteínas estructurales son; la de membrana (M), la de envoltura (E), la nucleocápside (N) que liga el ARN y conforma la cápside, la espicular "*spike protein*" (S) y, la hemaglutininaesterasa (HE). Las proteínas no estructurales son al menos 16 y son conocidas como nsp1 a nsp16.

Las estructurales forman la partícula viral, dentro de ellas, la proteína S es la más antigénica y externa, además de ser la responsable de la forma del virus. Esta se une a la célula del huésped con la entrada del virus secundaria. Está compuesta por las subunidades S1 (que favorece la adhesión) y S2 (responsable de la fusión a la membrana). El dominio receptor de unión "*receptor binding domain*" (RBD) es la fracción de la subunidad S1 que se une a la enzima convertidora de angiotensina 2 "*angiotensin-converting enzyme*" 2 (ACE2, por sus siglas en inglés) de la célula hospedadora. SARS-CoV-2 emplea esta enzima y la serina-proteasa TMPRSS2 para su penetración[9].

Se cree que la secuencia WIV04/2019 fue la primera en infectar a los humanos, y es conocida como "secuencia cero"[4]. Pero, el SARS-CoV-2 cambió y evolucionó en el tiempo dando lugar a las mutaciones o cambios en el ARN que son detectadas con la secuenciación genómica completa. Rambaut et al. propusieron que se empleen los linajes para los subtipos de SARS-

CoV-2 en un artículo de 2020 en Nature Microbiology[10]. Un linaje es un conjunto de variantes de virus muy parecidos desde el punto de vista genético derivados de un ancestro en común. Una variante tiene una o más mutaciones que la diferencian de las otras. Hasta diciembre de 2020, se habían identificado dos linajes raíz (A y B) de los que descienden otros con evidencia filogenética a los que se les asigna un valor numérico (ej. linajes A.1 o B.2). A principios de 2020, B.1 es el linaje global predominante, subdividido en más de 70 sublinajes.

La OMS[11] distingue entre variantes de interés (VOI, por sus siglas en inglés "Variant of Interest") y las variantes preocupantes (VOC, por sus siglas en inglés "Variant of Concern") según las repercusiones que tengan en la salud pública respecto a la transmisibilidad, la virulencia, la gravedad de la enfermedad y disminución de la eficacia de las vacunas y/o tratamientos disponibles o medios de diagnóstico. La siguiente tabla es una elaboración propia basada en la evidencia científica de las variantes del COVID-19[12-13].

Tabla 1. Resumen de los tipos de variantes de interés (VOI) y preocupantes (VOC) descritos hasta la actualidad.

Nomenclatura de la OMS	**Linaje**	**Mutaciones de la proteína S (espiga)**	**País donde se descubrió el 1º caso**	**Mes y año de la detección del 1º caso**	**Motivos de preocupación**
Alpha (VOC)	B.1.1.7 501Y.V1	N501Y D614G P681H	Reino Unido	Septiembre 2020	Mayor transmisión (Ro) y severidad.
Beta (VOC)	B.1.351 B.1.351-2 B-1-351.3 501Y.V2	K417N, E484K, N501Y, D614G	Sudáfrica	Octubre 2020	Mayor transmisión y severidad. Reduce la inmunidad previa (adquirida por vacunación o infección previa).
Gamma (VOC)	P.1 P.1.1 P.1.2	K417T, E484K, N501Y, D614G	Brasil	Enero 2021	Mayor transmisión. Reduce la inmunidad

	501Y.V3				previa y el efecto de los tratamientos monoclonales.
Delta (VOC)	B.1.617.2 AY.1 AY.2	L452R, D614G, P681R	India	Diciembre 2020	Mayor transmisión. Reduce la inmunidad previa y el efecto de los tratamientos monoclonales.
Kappa (VOI)	B.1617.1	G142D, E154K, L452R, E484Q	India	Diciembre 2020	Mayor gravedad. Reduce el efecto de los tratamientos.
Eta (VOI)	B.1.525	Q52R, A67V, 144del, E484K, D614G	Reino Unido y Nigeria	Diciembre 2020	Reduce el efecto de la inmunidad previa y de los tratamientos monoclonales.
Iota (VOI)	B.1.526	L5F, T95L, D253G, D614G	EE. UU. (New York)	Noviembre 2020	Reduce el efecto de la inmunidad previa y de los tratamientos monoclonales.
Epsilon (VOI)	B.1.427 B.1.429	S131,W152C, L452R, D614G	EE. UU.(California)	Septiembre 2020	Reduce el efecto de la inmunidad previa.
Lambda (VOI)	C.37	L452Q,F490S ,D61G	Perú	Diciembre 2020	Reduce el efecto de la inmunidad previa.

Zeta (VOI)	P.2	E482K, D614G, V1176F	Brasil	Noviembre 2020	Reduce el efecto de la inmunidad previa.
Theta (VOI)	P.3	E484K, N501Y, D614G, P681H	Filipinas	Enero 2021	Aumento de transmisión y gravedad.
Mu (VOI)	B.1.621	R346K, E484K, N501Y, D614G, P681H	Colombia	Enero 2021	Mayor transmisión. Menos efecto de la inmunidad previa.
Ómicron (VOC)	BA.1.1.529 BA.2 BA.3 BA.4 BA.5 BQ.11 XBB Y sus sublinajes desdendientes	L452R, F486V, R493Q	Botsuana/Sudáfrica	Noviembre 2021	Mayor transmisión. Reduce el efecto de la inmunidad previa. Disminuye efecto anticuerpos monoclonales.

4.1.2. Fisiopatología.

Interacción con el Sistema resina angiotensina-aldosterona.

El nuevo SARS-CoV-2 utiliza el receptor enzima convertidos de angiotensina 2 (ECA2) como el SARS-CoV original para la entrada del virus en las células conllevando una disminución de estos receptores y una mayor producción de angiotensina-2 (AT2). La función de la ECA2 es la transformación de la Angiotensina I en Angiotensina 1-9 y de la Angiotensina II en Angiotensina 1-7. Estos productos finales tienen efectos vasodilatadores, antifibróticos, antiinflamatorios y favorecedores de la natriuresis. Así, todos reducen la tensión arterial, contrarregulando la acción de la AT2. Aproximadamente 83% de los receptores de la AT2 se expresan en las células epiteliales alveolares de tipo II, responsable se producir el surfactante pulmonar y progenitor de los neumocitos tipo I en caso de lesión tisular. No obstante,

también se encuentran en diferentes tejidos como el miocardio, el riñón, las vías respiratorias y a nivel vascular[14-15].

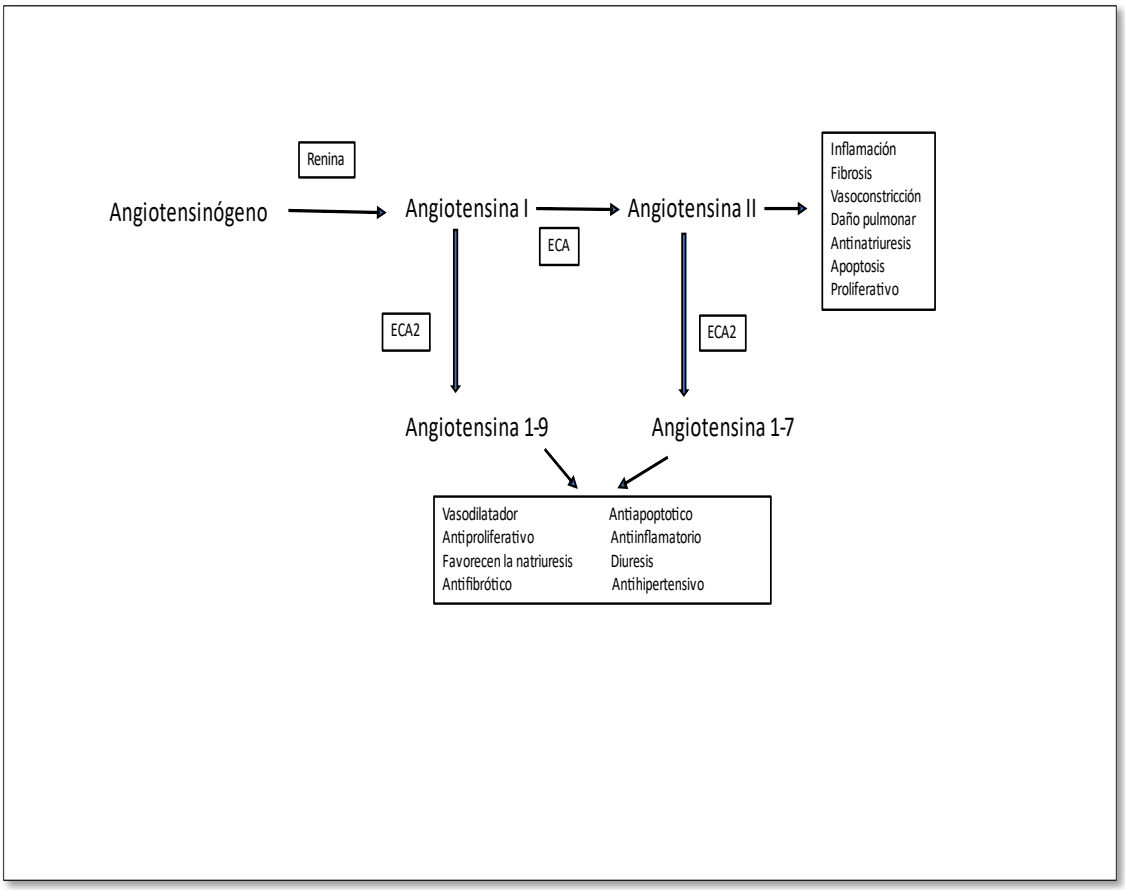

Figura 4. Papel del SARS-CoV-2 con el sistema renina angiotensina-aldosterona.

Interacción con el sistema inmunitario.

La inmunidad juega un papel primordial en la determinación de la gravedad y del curso del COVID-19.

1. *Respuesta inmune innata.*

La infección por SARS-CoV-2 activa inicialmente las vías inmunitarias innatas que inducen activación potente de la vía del interferón (IFN) de tipo I/III, la producción de citocinas proinflamatorias y el reclutamiento de neutrófilos y células mieloides actuando como factor iniciador de la respuesta inmunitaria. Así, el sistema inmune innato y la forma en que se activa inicialmente es el que determina el tipo de respuesta contra el virus SARS-CoV-2. Si la activación es moderada, inducirá una respuesta temprana y eficiente de interferón tipo I, se atraerá al sistema inmune adaptativo y habrá inflamación moderada que contribuirá a la erradicación de las células infectadas por virus y reparación posterior del daño. Cuando la

respuesta del interferón es tardía o insuficiente y el daño tisular causado por el virus es muy grande, o la respuesta inflamatoria es desproporcionada, se genera una señal de daño muy grande que generará un estado hiperinflamatorio local, que generará una activación desproporcionada del sistema inmune adaptativo y el cuadro descrito de síndrome de activación macrofágica. Los interferones tipo I inhiben la síntesis de proteína viral, la expresión de genes, ensamblaje de viriones e inducen la degradación del ARN del virus[16].

El virus SARS-CoV-2 se une y penetra al epitelio respiratorio a través del receptor en la superficie de la célula epitelial ECA2 como hemos explicado anteriormente, el cual es más predominante en los adultos y, sobre todo, en varones; de ahí que sea el grupo más afectado. Al unirse a su receptor, el virus penetra la célula y es reconocido por los receptores de reconococimiento de patrones (PRR) de los macrófagos. Dentro de estos receptores se distinguen dos grupos; los que reconocen patrones moleculares asociados con patógenos (PAMPS) que son principalmente los receptores tipo toll (TLR) y, los receptores que reconocen los patrones asociados a los daños (DAMP). La vía de señalización de los TLR induce en la célula infectada la producción de citocinas pro-inflamatorias, como el factor de necrosis tumoral alfa (TNFa) y la interleucina-6 (IL-6) y, también, la producción de interferones de tipo inflamatorio o tipo I (a y b)[17]. Todo esto conlleva cambios en la microcirculación local, con activación del endotelio y salida de células del sistema inmune (células NK, monocitos, linfocitos y neutrófilos) activando a los macrófagos y células dendríticas, que tendrán un perfil inflamatorio y contribuirán a la producción de más citocinas inflamatorias que pueden disparar una respuesta de hiperinflamación, responsable de las formas graves de la enfermedad[18].

Esta hiperactivación se ha denominado síndrome de liberación de citoquinas (CRS, por sus siglas en inglés), que estaría asociada al síndrome de insuficiencia respiratoria aguda o Síndrome de Distrés Respiratorio del Adulto (SDRA) que se ha descrito como la principal causa de mortalidad por COVID-19[19].

2. *Respuesta inmune adaptativa.*

El sistema adaptativo consta de tres componentes principales: las células B (respuesta humoral con producción de anticuerpos), las células T colaboradoras $CD4^+$ y las células T citotóxicas $CD8^+$ (respuesta celular). El armamento de células B, células T $CD4^+$ y células T $CD8^+$ tiene diferentes funciones en diferentes infecciones virales. En condiciones normales, las células B producen anticuerpos, las células T $CD4^+$ poseen una gama de funcionalidades auxiliares y efectoras y, las células $CD8^+$ matan las células infectadas. En la enfermedad

covid-19 los pilares principales de esta respuesta adaptativa antiviral efectora son los anticuerpos neutralizantes (NAbs), que evitan que el virus infecte las células, y las células T (linfocitos T) citotóxicas (CD8 +), que pueden eliminar selectivamente las células infectadas. Las células T colaboradoras (T CD4 +) específicas frente al virus, constituyen el tercer pilar y son fundamentales, puesto que coordinan la reacción inmunitaria[18].

Sin embargo, el SARS-COV-2 provoca comportamientos inadecuados que conllevan un retraso en la activación y respuesta de los linfocitos T y un aumento de la replicación viral aumentando la gravedad de la enfermedad. Esta ausencia podría provocar una repoblación de células innatas más agresivas que amplifican la inflamación de los pacientes graves.

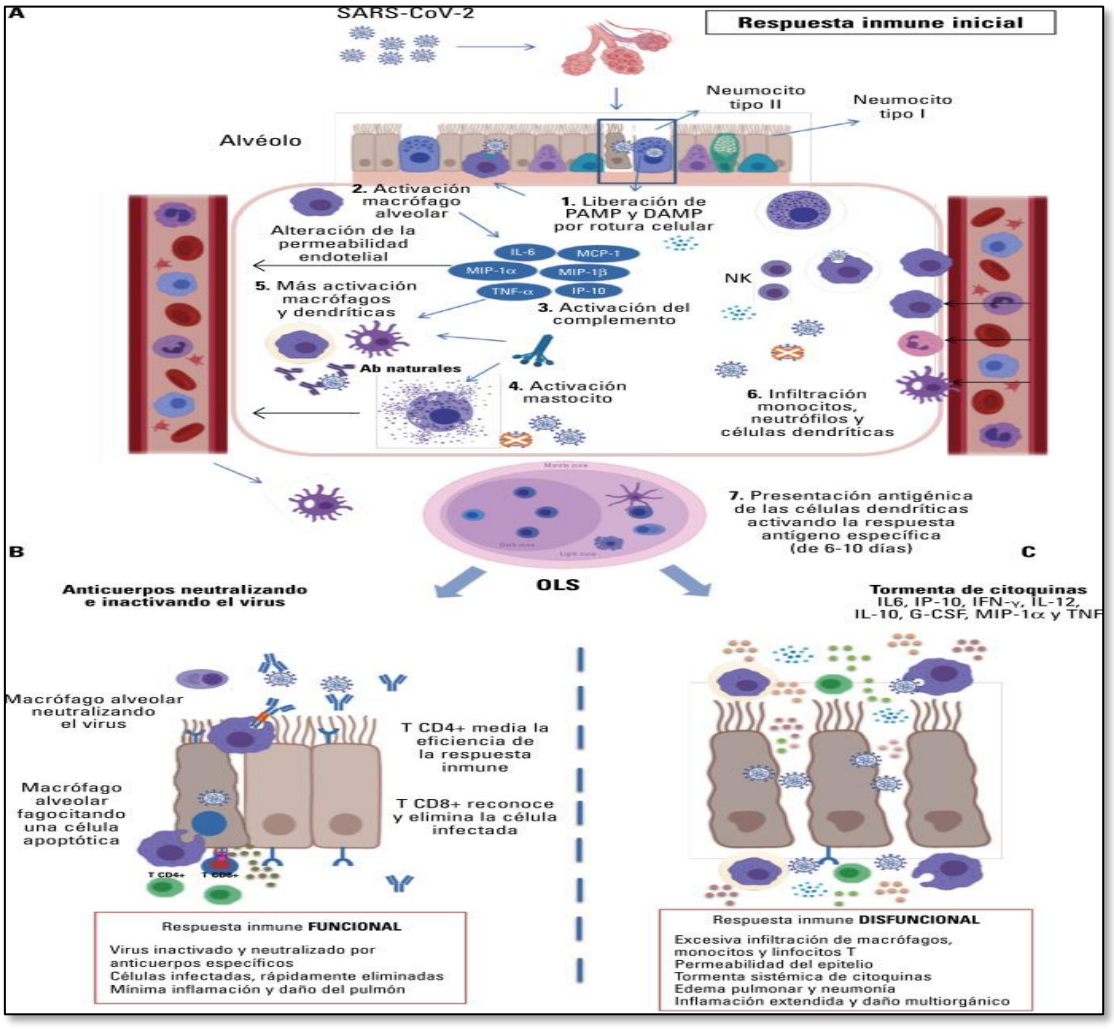

Figura 5. Sistema inmunológico en la infección por el SARS-CoV-2: Inmunopatología de la COVID-19. Tomada de Sanz M[18].

Los pacientes con COVID-19 presentan una linfopenia T circulante característica[20], siendo más acusada en los pacientes severos y críticos que en los pacientes moderados, y no parece que sea diferencial entre linfocitos Th y Tc. Además, esta linfopenia es proporcional a la

elevación de los niveles de IL-6 y a la inadecuada respuesta del compartimento T que aparece en los pacientes graves.

Interacción con la coagulación y el sistema microvascular.

La activación excesiva del sistema inmune innato causa la descrita tormenta de citoquinas la cual desencadena daños a nivel microvascular y activa el sistema de coagulación con inhibición de fibrinolisis. Así, la enfermedad grave por SARS-CoV-2 se asocia a un estado de hipercoagulabilidad. Hay unas diferencias entre la coagulación intravascular diseminada (CID) producidas por la sepsis y, la coagulopatía asociada al SARS-CoV-2; la trombocitopenia asociada a la COVID-19 es leve y hay un consumo escaso de los factores de coagulación.

Otras características descritas de la coagulopatía asociada al SARS-CoV-2 son[21]:

1. La coagulopatía asociada a la COVID-19 se caracteriza por elevación del fibrinógeno y del dímero D (DD), discreta elevación del tiempo de protrombina (TP) y el tiempo de protrombina parcial activado (TTPa), y leve trombocitopenia en las primeras etapas de la enfermedad.
2. La coagulopatía parece estar relacionada con la gravedad de la enfermedad y la inflamación asociada, y no con la actividad viral intrínseca.
3. Los niveles de IL-6 muestran una correlación positiva con el aumento del fibrinógeno.
4. El aumento progresivo del DD durante la hospitalización se asocia con fallo multiorgánico y CID
5. El DD elevado al ingreso se asocia con una mayor mortalidad.
6. Los datos sobre las manifestaciones hemorrágicas en la COVID-19 son muy escasos, aunque parecen no ser comunes a pesar de la coagulopatía.

4.1.3. Fuentes de infección SARS-CoV-2 y transmisión.

Desde el principio se ha propuesto un origen zoonótico, es decir, puede transmitirse de los animales a los humanos. Con los estudios filogenéticos publicados se propone que el SARS-CoV-2 se originó del linaje de un virus de murciélago, con el que tiene un 96% de similitud[22]. No obstante, parece que puede existir un huésped intermedio entre el murciélago y el ser humano, donde este virus se ha adoptado a nuestra especie habiéndose propuesto el pangolín[23] entre otros. Entre los mecanismos de transmisión distinguimos:

Mecanismo de transmisión animal-humano.

En literatura reciente se ha documentado cómo el epicentro de la pandemia fue el mercado de Wuhan donde además de vender mariscos, aves y otros productos básicos, se vendieron

zorros rojos, tejones porcinos, y perros mapaches comunes[24]. No se sabe que haya informes disponibles para los resultados de las pruebas de SARS-CoV-2 de estos mamíferos en el mercado, pero si existen muestras ambientales positivas para el virus. Aunque el modo exacto de transmisión del virus de la fuente animal a la humana no se conoce, todo apunta a vía digestiva por consumo de animales infectados o por sus secreciones[25].

Mecanismo de trasmisión humano-humano.

El virus se transmite generalmente de persona a persona a través de pequeñas gotas de saliva, conocidas como gotas de Flugge, que se emiten al hablar, estornudar, toser o respirar. También está documentada la transmisión por aerosoles (< 5µm). Se difunde principalmente cuando las personas están en contacto cercano, pero también se puede difundir al tocar una superficie contaminada y luego llevar las manos contaminadas a la cara o las mucosas. La transmisión vertical a través de la placenta es posible, aunque infrecuente. Otras vías de transmisión son muy improbables[26].

4.2 COVID-19.

El acrónimo SARS-CoV-2 hace referencia al virus (por las siglas en inglés "*severe acute respiratory syndrome-related coronavirus*") responsable de la enfermedad denominada COVID-19 ("*coronavirus disease*" 2019) como ya hemos descrito anteriormente.

4.2.1. Periodo de incubación y duración de la enfermedad.

El período de incubación de una enfermedad está descrito como la duración entre la exposición inicial y el inicio de los síntomas de la enfermedad. La mediana del periodo de incubación del SARS-CoV-2 es de 5,1 días (IC 95% 4,5 a 5,8), con un rango de 1 a 14 días. Se puede ser contagioso desde 1-2 días antes de la aparición de la clínica y hasta 5-6 días después. No obstante, se han descrito casos con transmisión viral hasta 30 días tras el contagio en pacientes asintomáticos [27].

Cuando la enfermedad cursa de forma leve el tiempo medio desde el inicio de la clínica hasta la recuperación es de 2 semanas y cuando lo hace de forma grave es de 3-6 semanas. En los casos críticos la hipoxemia con el desarrollo del SDRA suele instaurarse sobre el 8º día y de 2-8 semanas hasta que se produce el fallecimiento.

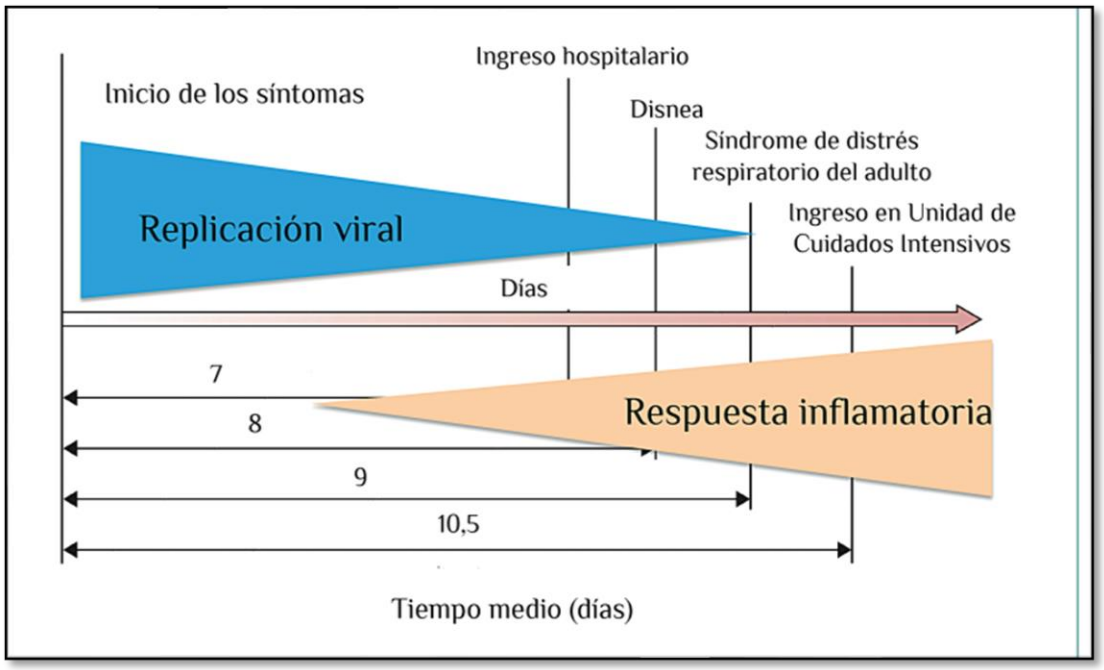

Figura 6. Fases de la infección SARS-CoV-2. Tomada de Sanz I[5].

La detección prolongada del ARN viral en el tiempo no implica una mayor infecciosidad. Esta condición de duración de la eliminación del ARN es variable y puede estar influenciada por mayor edad y gravedad[28-29].

4.2.2. Tasa de ataque secundario, número básico (R0) y efectivo (Re) de reproducción y factor de dispersión K.

La *tasa de ataque secundario (TAS)* representa el número de casos de una enfermedad que aparecen dentro del periodo de incubación entre los contactos susceptibles. Varía según las condiciones en las cuales se produzca la transmisión: el tipo y la duración de la exposición, el ambiente cerrado o abierto, etc. En los estudios pareados de casos y contactos cercanos la TAS para COVID-19 ha sido muy variable situándose entre el 0,7% y el 75%.

El *número básico de reproducción R0* es el promedio de casos secundarios producidos a partir de un caso en ausencia de medidas de prevención. Dos revisiones recogen un total de 32 estudios estimando un R0 de entre 1,5 y 6,5 durante la epidemia de Wuhan.

Pero no todos los casos contribuyen de la misma forma a la transmisión de la enfermedad. Por esto, hay que considerar el *factor de dispersión k* que representa la variación con la que se distribuyen los casos secundarios a un caso conocido, pues esto no depende exclusivamente de la contagiosidad del caso índice, sino que, algunos casos a pesar de tener

una R0 de 2-3 no producirán ningún caso secundario, otros producirán un número pequeño de casos secundarios y viceversa. Este fenómeno es lo que se conoce como evento superdiseminador y como vemos, esta influenciado por los contactos estrechos e intensos.

Por último, el *número reproductivo efectivo (Re)* es la estimación de cuantas personas en promedio se han contagiado cada día a partir de los casos existentes observados durante una epidemia (en el momento que son notificados), por tanto, Re es un valor que varía en función de la evolución renal de la epidemia y permite seguir su dinámica progresiva[30].

En la figura 7 se puede observar la evolución de la Re en España, durante el primer periodo de la epidemia en el que el esfuerzo de la Salud Pública se centró en la contención hasta mediados de marzo 2020. En la segunda fase tras la declaración de pandemia mundial, se adoptaron medidas de distanciamiento social más intensas progresivas hasta el confinamiento de la población (excepto algunos sectores laborales imprescindibles), observando como la Re va descendiendo debido a la efectividad de estas medidas.

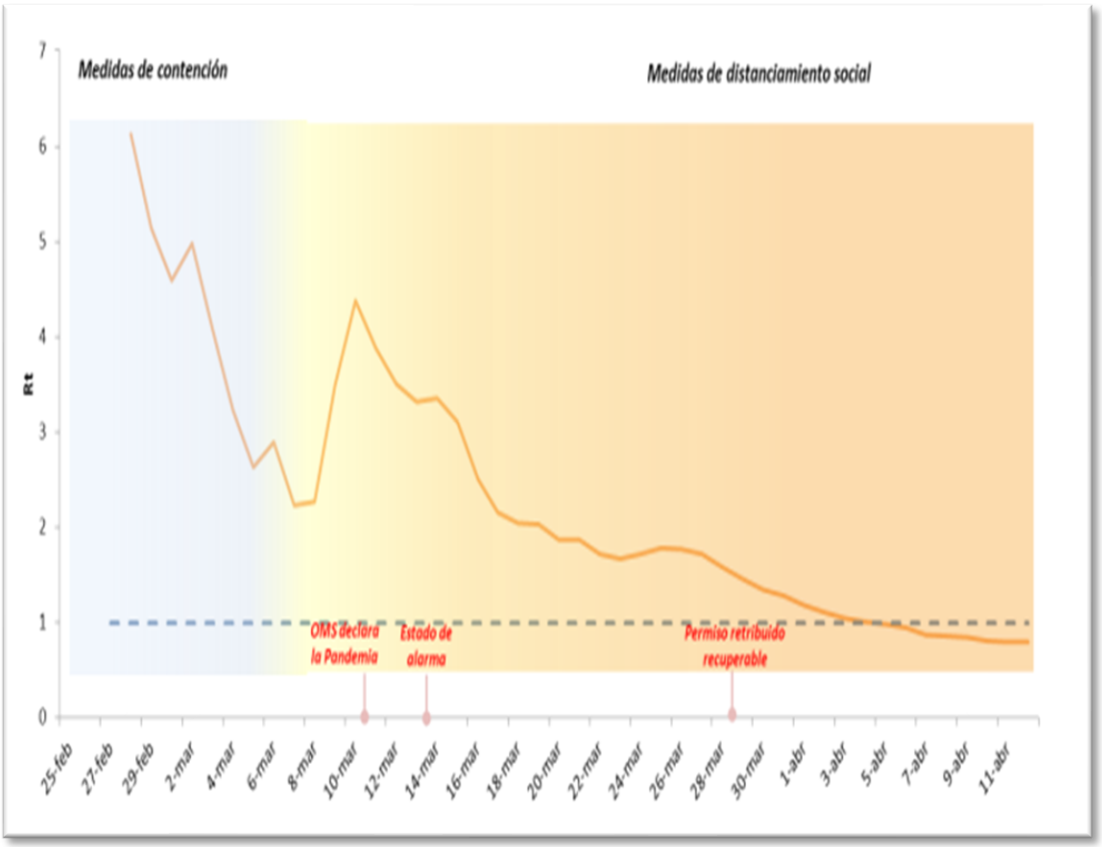

Figura 7. Número de reproducción efectivo (Re) en España desde el 25 de febrero hasta el 12 de abril de 2020 con medidas de control implementadas durante la pandemia. Tomada de parámetros epidemiológicos del ministerio de sanidad[24].

4.2.3. Gravedad y letalidad.

La *gravedad* de una enfermedad depende de los criterios que se establezcan para cada enfermedad y de la capacidad epidemiológica para diagnosticarlos. Por ello, ante una enfermedad nueva y desconocida como el COVID-19, los criterios no estaban claros inicialmente. La gravedad de una enfermedad esta influenciada por tres tipos de factores; del agente causal (virulencia), factores extrínsecos (demográficos, tratamientos y vacunas, acceso y calidad de asistencia sanitaria) y factores intrínsecos de la persona (susceptibilidad). En enfermedades emergentes, al ser los primeros casos diagnosticados por un curso sanitario grave, el primer conocimiento de la enfermedad da una visión de mayor gravedad, la cual va descendiendo conforme se diagnostican casos más leves.

La misma situación pasa con la *letalidad*, pues se calcula a partir de los fallecimientos producidos por los casos confirmados de una enfermedad, estando así influenciada por la capacidad del sistema para declarar y detectar los casos que fallecen (numerador) y, por la capacidad de diagnosticar y confirmar todos los casos de la enfermedad (denominador). La tasa de letalidad a nivel mundial es de aproximadamente el 4 %, pero varía en función de las características demográficas de la población local. Así, al inicio de una enfermedad emergente pueden detectarse sólo los casos más graves, estimando una letalidad más superior que la real. Los datos al inicio de las epidemias o durante los periodos de mayor intensidad deben tomarse con cautela ya que probablemente luego cambien.

Respecto al exceso de muertes durante la pandemia, la tasa de letalidad no representa esta cifra, pues este exceso de mortalidad esta influenciado también por otros factores como la demora en la atención, el sistema sanitario sobrecargado y ciertos determinantes sociales de salud[30].

4.2.4. Manifestaciones clínicas.

El COVID-19 es una enfermedad multisistémica siendo su presentación clínica amplia y diversa.

Un 40-45% de los infectados son asintomáticos en el momento del diagnóstico viral. Un 80 % de los infectados tendrán una enfermedad leve; el 15 % desarrollarán una enfermedad más grave (con disnea, hipoxia o >50 % de afectación pulmonar en los estudios por imágenes) requiriendo hospitalización, y otro 5 % con enfermedad crítica (insuficiencia respiratoria, arrítmicas, sangrados gastrointestinales, sepsis, shock o disfunción multiorgánica) con necesidad de ingreso en la UCI[31].

Los sujetos aun siendo asintomáticos pueden presentar alteraciones radiológicas según se ha evidenciado en la literatura. De hecho, muchos se han diagnosticado casualmente por los hallazgos de estas pruebas.

Los síntomas más frecuentes son fiebre, escalofríos, fatiga, tos seca, anorexia, mialgias, diarrea y producción de esputo. También se informan con frecuencia pérdida del olfato (anosmia) y pérdida del gusto (disgeusia)[32]. El dolor de garganta, la congestión nasal y la rinorrea son menos frecuentes. A continuación, vamos a describir la sintomatología que puede aparecer según el órgano afecto[13], así como los mecanismos etiológicos que la subyacen:

1. Manifestaciones pulmonares: Pueden ser leves, moderadas o graves. Los casos leves desarrollan tos o dolor de garganta y, pueden evolucionar a moderados con neumonía o graves con SDRA. Las hipótesis para el desarrollo del distrés pulmonar son; 1) Debida a la unión del virus con los receptores ECA2, se produce una destrucción y daño en las células alveolares que produce una disminución del surfactante pulmonar con el aumento de la tensión superficial del pulmón y predisposición al SDRA; 2) La segunda hipótesis se basa en el desarrollo de la tormenta de citoquinas.
2. Manifestaciones cardiovasculares: Desde arritmias, debut de hipertensión arterial (HTA), palpitaciones, miocarditis, pericarditis, síndrome coronario agudo (SCA), insuficiencia cardiaca, shock cardiogénico hasta paro cardiaco. Mecanismos etiológicos: 1) El efecto del SARS-CoV-2 sobre los receptores ECA2 en el tejido cardiaco. 2) Tormenta de citoquinas que puede causar insuficiencia y otras manifestaciones cardiacas. 3) Algunos medicamentos que se usaron para tratar el covid-19 podían desencadenar arrítmicas, en concreto, la hidroxicloroquina y la cloroquina. 4)Hipoxia por el SDRA que conduce a disminución de oxígeno al músculo cardiaco, conllevando infarto agudo de miocardio (IAM).5) Lesión vascular debida al efecto del SARS-CoV-2 en las células endoteliales que conducen a la agregación plaquetaria y placas vasculares o trombos que pueden inducir IAM[33].
3. Manifestaciones gastrointestinales: Estreñimiento, anorexia, náuseas, vómitos, dolores abdominales y gástricos, lesiones pancreáticas y hepáticas. Su mecanismo explicativo se basa en 3 teorías; 1) La sobrecarga vírica y el efecto directo del SARS-CoV-2 sobre los receptores ECA2 en el epitelio del esófago, estómago, intestino delgado e hígado. 2)La tormenta de citoquinas puede inducir síntomas de

gastroenteritis, y lesiones orgánicas del hígado y/o páncreas, 3) Medicamentos como antivirales que son hepatotóxicos[34].

4. Manifestaciones renales: La insuficiencia renal aguda (IRA), la hematuria y la proteinuria han sido descritas como complicaciones renales de la COVID-19. Mecanismo etiológico: La sobrecarga viral hace que el receptor ECA2 que se encuentra en los túbulos renales se vea afectado, lo cual empeora con la tormenta de citoquinas desencadenando la insuficiencia renal.

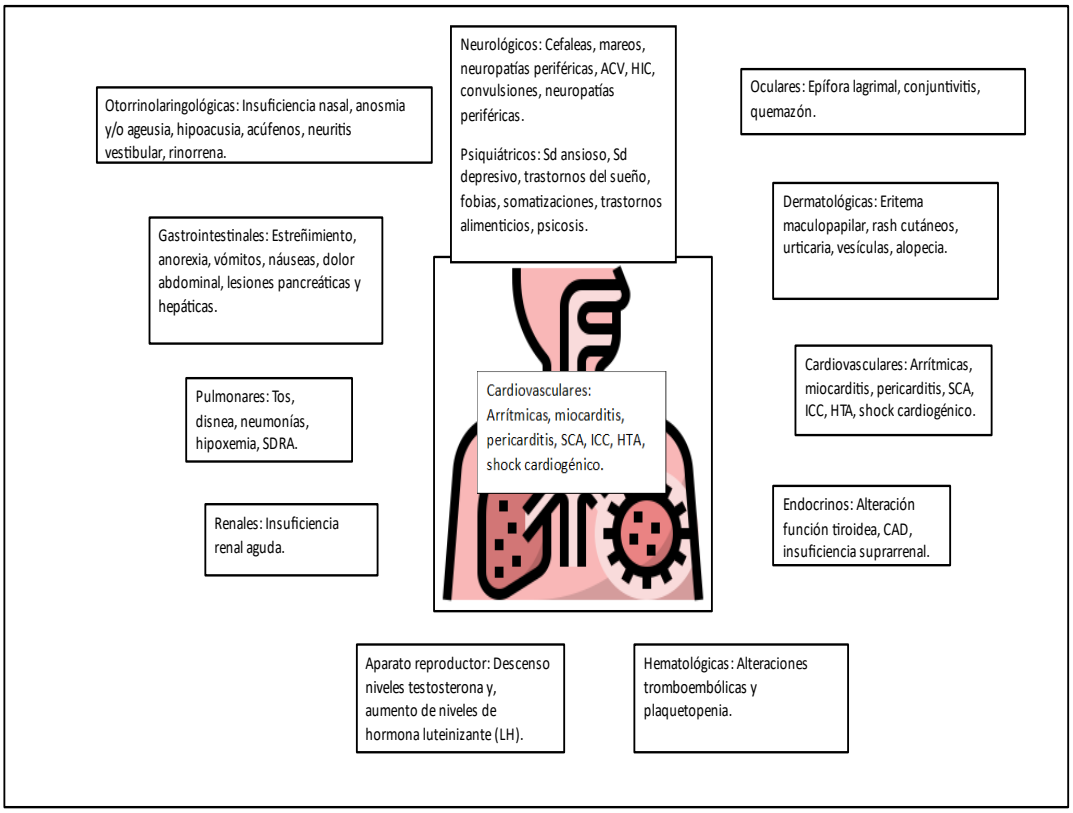

Figura 8. Manifestaciones sistémicas del COVID-19.

5. Manifestaciones neurológicas: Pueden ser síntomas centrales o periféricos según las lesiones sean de los nervios centrales o periféricos. Las manifestaciones centrales varían desde la cefalea, mareos, accidente cerebrovascular isquémico (ACV), hemorragia intracraneal encefalitis, convulsiones, anosmia o insomnio. Las manifestaciones de los nervios periféricos pueden incluir neuropraxia, oftalmoplejía, ataxia, síndrome de Miller Fisher, síndrome de Guillain-Barre y, pérdida del reflejo tendinoso. También se han notificado casos de mielitis transversa aguda con

hipotonía en algunos pacientes. Los mecanismos etiológicos son los siguientes: 1) El efecto directo del SARS-CoV-2 sobre los receptores del cerebro y nervios;2) La hipoxia puede inducir daño a nivel cerebral y conllevar edema de este;3) La tormenta de citoquinas;4) El efecto tromboembólico del COVID-19 que puede desencadenar isquemia cerebral y ACV[35-41].

6. Manifestaciones psiquiátricas: Pueden aparecer síntomas psiquiátricos como resultado del aislamiento social y cuarentena de la enfermedad, manifestándose como cuadro depresivo, ansiedad, trastornos del sueño, trastornos alimenticios, somatizaciones y fobias. También, el propio SARS-CoV-2 puede afectar al tejido cerebral y aparecer trastornos psiquiátricos como psicosis, resultado del efecto del propio COVID-19[42].
7. Manifestaciones dermatológicas: Eritema maculopapular, enrojecimiento y erupción cutánea. El proceso fisiopatológico se basa; 1) El efecto del SARS-CoV-2 sobre la ECA2 en la capa basal de la piel; 2) El efecto producido por la tormenta de citoquinas; 3) Reacciones secundarias a fármacos[43-44].
8. Manifestaciones oftalmológicas: Sensación de quemazón ocular, ojo seco, conjuntivitis, lagrimeo, enrojecimiento. Las manifestaciones oculares pueden deberse al; 1) Efecto directo del virus que se transmite por gotitas a la córnea y conjuntiva; 2) El virus presente en las gotitas se une a los receptores ECA2 ubicados en la córnea y conjuntiva.
9. Manifestaciones otorrinolaringológicas: Obstrucción nasal, disfunción olfatoria o del gusto, rinorrea, neuritis vestibular, hipoacusia, acúfenos, epistaxis. Mecanismos posibles; 1) El efecto del SARS-CoV-2 sobre la ECA2; 2) El efecto producido por la tormenta de citoquinas.
10. Manifestaciones endocrinas: Alteraciones en la función tiroidea, cetoacidosis diabética (CAD) en pacientes con DM2 o cetosis en pacientes sin diabetes, insuficiencia suprarrenal. Suelen ocurrir como manifestaciones tardías. La expresión de ECA2 se encuentra en glándulas como el tálamo, la tiroides, la hipófisis y el páncreas, lo cual explica los daños producidos por el COVID-19 en el sistema endocrino. También la tormenta de citoquinas influye en la disfunción endocrina por la insuficiencia multiorgánica que produce [45-46].
11. Manifestaciones del aparato reproductor: Descenso de niveles de testosterona y aumento de niveles de la hormona luteinizante. Mecanismos posibles: 1) La expresión de ECA2 en el tejido testicular y; 2) Tormenta de citoquinas[47-48].

12. Manifestaciones hematológicas: Alteraciones tromboembólicas y plaquetopenia. La ECA2 tiene receptores en las células endoteliales, produciendo un efecto directo sobre la integridad de estas con posible desenlace en enfermedad tromboembólica venosa (ETEV), con trombosis venosa profunda (TVP) y tromboembolismo pulmonar (TEP) [49-50].
13. Complicaciones inflamatorias: Algunos pacientes de COVID-19 grave tienen analíticas con parámetros de laboratorio que evidencia una respuesta inflamatoria exagerada; Linfopenia, plaquetopenia, con aumento de lactato deshidrogenasa (DLH), dímero D (DD), tiempo de protrombina (TP), proteína C reactiva (PCR) y ferritina. También elevación de enzimas hepáticas; aspartato transaminasa (AST) y alanina aminotransferasa (ALT). La insuficiencia renal aguda que puede ocurrir conlleva elevación de la urea, creatinina y nitrógeno ureico en sangre. Aunque estas alteraciones se han comparado con las presentes en el síndrome de liberación de citocinas, los niveles son más bajos en el COVID-19.
14. Manifestaciones musculoesqueléticas: Dolores articulares, dolores musculares, debilidad muscular. Mecanismos posibles: 1) Efecto directo del virus sobre los receptores ECA2 en el músculo y corteza ósea; 2) Reacción inflamatoria de la tormenta de citoquinas.
15. Manifestaciones en edades pediátricas: La sintomatología más frecuente en niños es la fiebre, tos, bronquitis aguda, dolores abdominales, estreñimiento, vómitos, náuseas, dolores osteomusculares, congestión nasal, erupciones cutáneas, dolor de garganta, anosmia y ageusia y conjuntivitis. En los niños, la infección es afortunadamente poco frecuente y, cuando ocurre suele ser leve, aunque también se han descrito complicaciones como la enfermedad de Kawasaki y el síndrome de choque tóxico.

En otras poblaciones especiales, como mujeres embarazadas y lactantes, el enfoque preventivo, diagnóstico y de tratamiento de la enfermedad es parecido al resto de personas.

4.2.5. Factores de riesgo/comorbilidades.

Los factores de riesgo para desarrollar una enfermedad grave están influenciados por la edad, presencia de comorbilidades, algunas características demográficas y las alteraciones de laboratorio marcadores que marcan un mal pronóstico[51].

1. Edad: Los casos graves se concentran en personas mayores. Aun así, no se ha podido establecer un umbral a partir del cual el riesgo esté aumentado, puesto que influyen

otros factores como la presencia de otras comorbilidades, los roles genéticos, el origen étnico y la vida en residencias cerradas.

2. Factores genéticos del huésped: Siguen siendo estudiados. No obstante, ser del grupo sanguíneo A se ha asociado con mayor riesgo. El sexo masculino junto con las poblaciones negras, hispanas y/o del sur de Asia también se ha asociado con peor evolución y pronostico.
3. Comorbilidades: La presencia de enfermedad cardiovascular, diabetes mellitus (DM), enfermedades pulmonares crónicas, neoplasias activas, enfermedad renal crónica, trasplantando de órganos sólitos o células madre, obesidad y ser fumador[52] influyen en la mala evolución.

El embarazo al inicio de la pandemia no se consideró factor de riesgo para la enfermedad covid-19, pero actualmente tras múltiples publicaciones se considera que es una situación de riesgo de complicaciones de la enfermedad y constituye un mayor riesgo para el curso del embarazo. También se ha demostrado que la infección influye en la prematuridad, la morbimortalidad perinatal y la morbilidad neonatal siendo estas más frecuentes.

El centro de control y prevención de enfermedades (CDC por sus siglas en ingles "center for diseases control and prevention") describe unos factores de riesgo (FR) asociadas a gravedad clasificados según niveles de evidencia[53]; 1) FR establecidos: Constituidos por aquellas condiciones médicas avalados por un metaanálisis o una revisión sistemática publicada. 2)FR probables**:** No tienen un metaanálisis publicado o una revisión sistemática que las respalde, así como tampoco han completado el proceso de revisión sistemática de los CDC. La evidencia está respaldada principalmente por estudios de cohortes, de casos y controles o transversales. 3)FR posibles: Aquellas condiciones cuyo metaanálisis o revisión sistemática no es concluyente, ya sea porque los datos agregados son inconsistentes o porque son insuficientes para la asociación, requiriendo más estudios (Ver tabla 2).

Tabla 2. Tabla de los factores de riesgo descritos por CDC de COVID-19 grave. Tomada de Coronavirus Disease 2019 [53].

Factores de riesgo establecidos	**Probables factores de riesgo**	**Posibles factores de riesgo**
Enfermedad cerebrovascular Asma Bronquiectasias	Sobrepeso (IMC ≥25 kg/m ^{2}, pero <30 kg/m ^{2})	Deficiencia de alfa 1 antitripsina

Cáncer	Anemia drepanocítica	Displasia broncopulmonar
Enfermedad renal crónica*	Trastornos por uso de sustancias	Hepatitis B
Enfermedades pulmonares crónicas:		Hepatitis C
-Enfermedad pulmonar intersticial		Hipertensión
-EPOC (enfermedad pulmonar obstructiva crónica)		Talasemia
-Embolia pulmonar		
-Hipertensión pulmonar		
Enfermedades hepáticas crónicas limitadas a:		
-Cirrosis		
-Enfermedad del hígado graso no alcohólico		
-Enfermedad hepática alcohólica		
-Hepatitis autoinmune		
Fibrosis quística		
Diabetes mellitus, tipo 1 y tipo 2*‡		
Malformaciones congénitas (defectos de nacimiento)		
Discapacidades Intelectuales y del Desarrollo		
Virus de la inmunodeficiencia humana (VIH)		
Afecciones cardíacas (como insuficiencia cardíaca, enfermedad de las arterias coronarias o cardiomiopatías)		
Trastornos de salud mental limitados a:		
Trastornos del estado de ánimo, incluida la depresión.		
Trastornos del espectro de la esquizofrenia		
Afecciones neurológicas limitadas a la demencia‡		
Obesidad (IMC ≥30 kg/m ^{2} o ≥percentil 95 en niños)*‡		
Inmunodeficiencias Primarias		
Embarazo y embarazo reciente		
La inactividad física		
Tabaquismo, actual y anterior		
Trasplante de órgano sólido o de células hematopoyéticas		
Tuberculosis		

Uso de corticosteroides u otros medicamentos inmunosupresores

* indica condiciones subyacentes para las cuales hay evidencia para personas embarazadas y no embarazadas

‡ afecciones subyacentes para las que existe evidencia en pacientes pediátricos.

En este contexto, es fundamental estudiar y profundizar en aquellos factores que han mostrado evidencias de estar relacionados con la recuperación funcional de los pacientes post-COVID como la proteína α1-antitripsina (AAT). La (AAT) es una glucoproteína perteneciente al grupo de las serpinas cuya función principal estriba en inhibir la elastasa del neutrófilo para prevenir la excesiva degradación proteolítica del tejido conectivo de los pulmones[54-55]. El déficit de α1-antitripsina (DAAT) es una enfermedad genética infrecuente que ocasiona niveles bajos defectuosos en sangre, aumentando el riesgo de desarrollar una variedad de enfermedades, que incluyen enfisema pulmonar y cirrosis hepática. Estudios recientes describen como dicha proteína posee funciones biológicas que pueden antagonizar tanto la infección por SARS-CoV-2 como los procesos fisiopatológicos subyacentes. Se ha postulado también que pacientes con alteraciones en su genotipado tienen mayor riesgo de gravedad e incluso muerte[56-57]. Además, en epidemias previas de otros coronavirus, un estudio de proteómica de 2004 informó que los pacientes que tenían niveles elevados de formas alteradas de AAT se correlacionaban con más gravedad del SRAS, y sugirió por esto, que las alteraciones de AAT podían ser biomarcadores del SRAS con una sensibilidad de 100 %[58].

4.2.6. Diagnóstico Virológico.

El diagnóstico de la infección se basa en la detección del virus mediante diferentes métodos directos (por reacción en cadena de la polimerasa (PCR), por su nombre en inglés Polymerase Chain Reaction y/o test rápidos de antígenos virales (RADTs), por sus siglas en inglés rapid antigen detection tests) y, métodos indirectos mediante test serológicos[59-61].

1. *Detección del material genético del virus:* Las pruebas de ampliación de ácidos nucleicos (NAAT) detectan el ARN del SARS-CoV-2, siendo la técnica de la PCR el tipo más común de NAAT. Este método de diagnóstico directo es el de elección, pues es el de mayor sensibilidad y especificidad en la actualidad (cercanas al 100%). Se basa en una técnica molecular de amplificación de ácidos nucleicos, es decir, material genético, por lo que, en el caso del ARN viral, es necesario primero convertirlo a ADN

(por transcriptasa inversa, RT, reverse transcription) para a partir de entonces iniciar el proceso de PCR (de ahí su nombre RT-PCR). Una vez que el genoma de interés es secuenciado, es necesario encontrar aquellas regiones únicas que lo diferencian de otros virus de la misma familia. En el caso del SARS-CoV-2 se buscan genes como el de la ARN polimerasa dependiente (RdRp), el gen E del virus y el gen N. Se pueden coger muestras del sistema respiratorio, así como de orina, heces e incluso sangre. Sus limitaciones son: 1) Requiere de personal especializado para realizar la técnica, pues se necesita que introduzca un hisopo en fosas nasales para extraer la muestra de la parte posterior nasofaríngea girando con suavidad durante 5-10 segundos, 2) El tiempo de resultado de 2-5horas y 3) Es relativamente costosa (10-15 euros por test sin considerar la instrumentación necesaria).

El umbral de ciclo (Ct) se refiere al número de ciclos de un ensayo de Rt-PCR necesario para amplificar el ARN viral para alcanzar un nivel detectable. Así, niveles de Ct más bajos reflejan niveles virales más altos.

En la actualidad se encuentran en desarrollo diversos sistemas rápidos de PCR (en menos de una hora), como por ejemplo el point of care de rRT-PCR que ha obtenido la a aprobación por la FDA de los EE.UU.

1. *Detección del virus como entidad individual,* es decir, *mediante antígenos virales:* En este caso, la detección es directa a través de las pruebas rápidas de antígenos virales (RADTs, rapid antigen detection tests). Las principales técnicas para la detección de antígeno-anticuerpo son los enzimoinmunoanálisis (ELISA) y la inmunocromatografía de flujo lateral (LFI). Los antígenos virales son proteínas que forman el virus y son detectadas por anticuerpos específicos que los capturan. Así, puede detectarse el virus completo a través de la proteína S o fragmentos de este por la proteína N. Entre sus ventajas incluye; obtener el resultado de forma rápida (5-15minutos); económica y bien establecida por el comercio. Presenta una sensibilidad entre el 20-40%, pero alta especificidad, es decir, alto valor predictivo positivo pues su positividad confirma el caso. Sus inconvenientes son; posibilidad de falsos negativos (FN), resultados cualitativos (positivo o negativo), problemas de reproducibilidad y necesidad de personal entrenado para la toma de las muestras por hisopos de exudado nasofaríngeo u orofaríngeo.
2. *Detección de los anticuerpos generados en el sujeto infectado (test serológicos*): Los test serológicos se basan en la detección indirecta del virus, a través de la

detección de anticuerpos generados por el propio organismo del sujeto. La IgM comienza aproximadamente a los 7 días desde el comienzo de la infección y empieza a disminuir tras la semana 4 de la misma y en gran parte de los casos es indetectable a partir de la tercera semana, aunque en algunos pacientes se ha visto hasta la 7º semana. De forma concomitante con la IgM, la IgG comienza un progresivo aumento desde la 3º semana hasta la 7º semana, indicando la activación de la respuesta humoral frente al coronavirus. La aparición de los diferentes tipos de inmunoglobulinas, junto con el resultado de la PCR, puede servir para el diagnóstico de la fase en la que se encuentra el enfermo. Como ventajas presenta ser rápida (5-15min), muestra de sangre capilar por tanto no invasiva adaptable a personal no especializado, con bajo coste (alrededor de 10-20 e por test). En la actualidad se disponen de pruebas comerciales y no comerciales que miden los anticuerpos aglutinantes utilizando diversas técnicas, entre ellas el inmunoensayo de flujo lateral (IFL), en ensayo de inmunoadsorción enzimática (ELISA) y el inmunoensayo de quimioluminiscencia (CLIA).

Según el Ministerio de Sanidad, en la fase inicial de la enfermedad, aproximadamente 1-7 días tras el inicio de los síntomas, es posible detectar tanto IgM como IgG y tener un resultado positivo en PCR. A partir de la segunda fase (8-14 días), es infrecuente detectar el virus en muestras respiratorias por PCR (aunque en algunos casos se prolonga por más tiempo) y, en la fase en la que el enfermo ya ha pasado su enfermedad y ha quedado inmunizado, es frecuente solo detectar IgG. Los antígenos se expresan sólo cuando el virus se replica activamente; por lo tanto, tales pruebas están diseñadas para identificar infecciones agudas o tempranas.

Las pruebas de diagnóstico arriba descritas no pueden confirmar la variante específica del SARS-CoV-2 que causa la infección. Se necesita secuenciación viral o pruebas de genotipado de PCR multiplex para hacer esto y, estos métodos no están disponibles de forma rutinaria para la toma de decisiones clínicas.

Tabla 3. Interpretación de las pruebas diagnósticas según fase de la enfermedad.

Interpretación	PCR	IgM	IgM	Antígeno
Fase preclínica	**+**	**-**	**-**	**-**
Fase inicial (1-7 días aprox)	**+**	**+/-**	**+/-**	**+/-**
2º fase (8-14 días)	**+/-**	**+**	**+/-**	**-**
3º fase (>15 días)	**+/-**	**+/-**	**+**	**-**
Infección pasada (memoria inmune)	**-**	**+/-**	**+**	**-**

4.2.7. Diagnóstico por imagen.

El diagnóstico temprano del COVID-19 se realiza mediante la tecnología de la PCR y/o determinación de anticuerpos en las pruebas serológicas como hemos expuesto en el apartado anterior. Sin embargo, estos diagnósticos microbiológicos no están exentos de resultados falsos negativos, motivo por el que la historia clínica y, las técnicas de imagen (radiografía de tórax y la TC también han sido una gran ayuda, sobre todo al inicio de la pandemia cuando los recursos microbiológicos eran limitados para el cúmulo de casos sospechosos[62-63].

La radiografía de tórax de los estadios iniciales de la enfermedad tiene un rendimiento diagnóstico limitado, ya que pueden no detectarse hallazgos patológicos que sí son identificables en la TC.

La TC es la técnica más sensible radiológica para definir los hallazgos encontrados en la neumonía COVID-19, pudiendo encontrarse afectación en vidrio deslustrado con o sin consolidaciones alveolares pulmonares, engrosamiento de los septos interlobulillares, bronquiectasias, etc. La afectación bilateral con predominio de lóbulos inferiores y distribución subpleural es la más frecuente descrita[64-65].

No obstante, existen otras modalidades de imagen además del TC que aportan información anatómica y funcional, como es la PET/TC que también se han utilizado en el diagnóstico y manejo de pacientes con COVID-19. La PET-TC es una modalidad de diagnóstico por imagen no invasiva, que permite la utilización de varios radiofármacos emisores de positrones capaces de evaluar diferentes procesos metabólicos y funcionales. El radiofármaco más empleado es el [18F]-FDG y representa un análogo de glucosa radiomarcado que se puede

utilizar para evaluar el metabolismo de la glucosa. Su captación celular por las células es proporcional a su consumo de glucosa y, como las células implicadas en la respuesta inflamatoria tienen una alta actividad glucolítica pueden ser identificados en sitios de inflamación e infección. Así, en enfermedades pulmonares inflamatorias e infecciosas puede ayudar a evaluar la carga viral, la severidad del daño agudo/crónico, la extensión pulmonar/extrapulmonar y, la valoración de la respuesta terapéutica, aunando la información morfológica (TC) y funcional (PET/TC)[66-67] que da. En este sentido, estudios previos han investigado la utilidad de la [^{18}F]FDG en el contexto de otras infecciones respiratorias en humanos causadas por coronavirus como el MERS-CoV y el SARS-CoV[68-69].

4.2.8. Vacunas.

Es fundamental entender cómo responde el sistema inmunológico adaptado al SARS-CoV-2 para entender la fisiopatología del virus, las complicaciones que desencadena, el desarrollo de tratamientos dirigidos y de vacunas en la actualidad.

Las vacunas actualmente vigentes frente al COVID-19 difieren entre sí por el mecanismo de acción que tienen para activar el sistema inmune pero tienen en común que actúan simulando una estructura en forma de espícula como es la proteína S[12-13]. Distinguimos 4 tipos:

1. *Vacunas con virus inactivados*; contienen virus completos que han sido inactivados o atenuados (a través del calor, radiación o productos químicos) para que sean inofensivos, pero que generen una respuesta inmune. Así incluyen cepas de SARS-CoV-2 inactivadas. Ejemplo de otras vacunas previas: poliomielitis y gripe. Compañías farmacéuticas: Sinopharm, Sinovac Biotech, Bharat Biotech.
2. *Vacunas de vectores víricos:* En este tipo de vacunas, el material genético de la COVID-19 se coloca en una versión modificada de otros virus (de ahí el nombre de vector vital) transfiriendo a las células instrucciones para fabricar antígenos de superficie (proteínas S) que estimula al sistema inmune. Ejemplo de otras vacunas previas: virus del ébola. Compañías farmacéuticas: AstraZeneca/Universidad de Oxford y Jannsen&Jannsen.
3. *Vacunas de ARN y ADN:* Estas vacunas introducen nanopartículas lipídicas que contienen ADN o ARNm. Este ARN mensajero incluye instrucciones para que nuestras propias células fabriquen proteínas S mostrándolas en su superficie (como las que se encuentran en la superficie del virus de la COVID-19) y estimulando el sistema inmunológico. Una vez se envíen estas señales, el ARNm o ADN se degrada

inmediatamente y no ingresa nunca en el núcleo celular. Compañías farmacéuticas: BioNTech/Pfizer, Moderna y CureVac.

4. *Vacunas basadas en antígenos peptídicos o subunidades:* Se utilizan fragmentos inofensivos de las proteínas S que imitan al virus COVID-19 con el objetivo de obtener una respuesta inmunitaria segura. No introduce el patógeno y no requiere de un vector viral. Ejemplo de vacunas previas: Heplisav-B, es decir, proteína de superficie del virus de la hepatitis B. Compañías farmacéuticas: Sanofi/GSK y Novavax.

Así, cuando una persona recibe la vacuna, el sistema inmune reconoce esta proteína S viral como un agente extraño y produce una respuesta humoral (anticuerpos neutralizantes) y celular (células citotóxicas) específica frente a la proteína S.

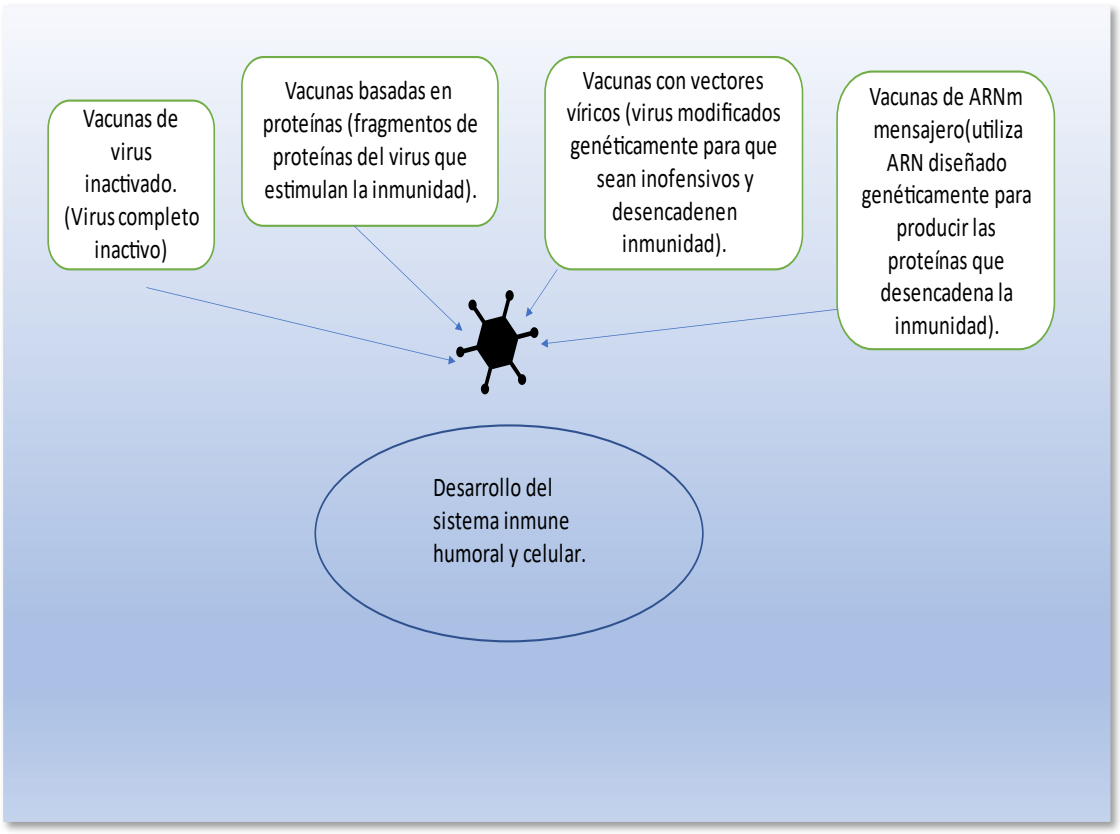

Figura 9. Mecanismos de funcionamiento de las vacunas frente al COVID-19.

El desarrollo de una vacuna requiere de un largo periodo de tiempo (4-7 años), pero en los tiempos de la pandemia los tiempos se acortaron. Distinguimos cuatro fases en el proceso de desarrollo; Ensayo clínico de Fase I: se comprueba la seguridad del fármaco; Ensayo clínico de Fase II: se empieza a comprobar si el fármaco funciona como se esperaba, se busca la

dosis más adecuada y el intervalo entre dosis; Ensayo clínico de Fase III: se verifican los aspectos de seguridad y eficacia del fármaco; Ensayo clínico de Fase IV: se examinan los efectos a largo plazo una vez el medicamento se ha comercializado con la población general. Estos estudios brindan información crucial sobre efectos raros o a largo plazo, la prevención de infecciones asintomáticas y la gravedad de COVID-19.

Tabla 4. Características principales de las vacunas incluidas en la OMS a fecha 12 de enero 2022.

	BNT162b2	**AZD1222**	**Ad26.COV2.S**	**ARNm-1273**	**BBIBP-CorV**	**Corona Vac**	**Covaxin**	**NVX-CoV22373**
Industria farmacéutica	Pfizer-Biontech	Oxfort &AstraZeneca	Janssen and Johnson &Johnson	Moderna	Sinopharm	Sinovac Biotech	Bharact Biotech	Novavax
Tipo de vacuna	mRNA	Vector viral (adenovirus de chimpancé)	Vector viral (adenovirus humano)	mRNA	Virus inactivo	Virus inactivo	Virus inactivo	Subunidad proteica
Antígeno objetivo	proteína S	Proteína S	Proteína S	proteína S	Virus entero	Virus entero	Virus entero	Protein a S
Almacenamiento	-75ºC	2-8ºC	2-8ºC	-75ºC	2-8ºC	2-8ºC	2-8ºC	2-8ºC
Nº dosis	2 con 28 días de diferencia	2 con 12 semanas de diferencia	1	2 con 28 días de diferencia	2 con 21 días de diferencia	2 con 14 días de diferencia	2 con 28 días de diferencia	2 con 21 días de diferencia
Población	≥18 años	≥18 años	≥18 años	≥18 años	≥18 años	≥18 años	≥18 años	≥18 años
Eficacia	94,1%	81,3%	66%	94,1%	78,1%	50,7%	77,8%	96,4%
Efectos secundarios más frecuentes.	Fiebre, cefalea, cansancio y dolor en la zona de inserción.	Fiebre, cefalea, dolor en la zona de inserción	Cefalea, cansancio, mialgias, náuseas, dolor en la zona de inserción.	Fiebre, cefalea, cansancio, dolor en la zona de inserción.	Fiebre, cefalea, cansancio y dolor en la zona de inserción.	Cefalea, cansancio y dolor en la zona de inserción.	Cefalea, cansancio, fiebre y dolor en la zona de inserción.	Cansancio, cefalea, dolor muscular, dolor en la zona de inserción y sensibilidad de

	la misma.

4.2.9. Tratamientos.

El tratamiento del COVID-19 se clasifica según la gravedad de la enfermedad. Se considera una enfermedad leve aquella que puede presentar todo tipo de sintomatología excepto la sensación disneica. Si esta sensación aparece hablamos de enfermedad al menos moderada y, estos pacientes casi siempre requieren de hospitalización. Consideramos una enfermedad grave cuando aparecen algunas de las siguientes circunstancias:

- Hipoxia (saturación de oxígeno menor de 94% en aire ambiente)
- Necesidad de aporte de oxígeno o soporte respiratorio.

Esquema de tratamiento:

1. *Pacientes no hospitalizados (enfermedad leve-moderada):* Se planteará un tratamiento si tiene los factores de riesgo reconocidos por la Agencia española de medicamentos y productos sanitarios (AEMPS)[70] o condiciones de alto riesgo de la CDC también expuestas anteriormente.

Factores de riesgo para progresión de COVID-19 según la AEPMS:

a) Personas inmunocomprometidas y con otras condiciones de alto riesgo, independientemente del estado de vacunación:
    - Receptores de trasplante de progenitores hematopoyéticos o CAR-T, en los dos años tras el trasplante/tratamiento, en tratamiento inmunosupresor o que tengan EICH independientemente del tiempo desde el TPH.
    - Receptores de trasplante de órgano sólido (menos de dos años o sometido a tratamiento inmunosupresor por sospecha de rechazo activo con independencia del tiempo desde el trasplante).
    - Tratamiento sustitutivo renal (hemodiálisis y diálisis peritoneal).
    - Inmunodeficiencias primarias: combinadas y de células B en las que se haya demostrado ausencia de respuesta vacunal.
    - Tratamiento activo con quimioterapia mielotóxica para enfermedades oncológicas o hematológicas. Se excluye el uso de hormonoterapia, inhibidores de checkpoint inmunes u otros tratamientos que no condicionan aumento en el riesgo de infección (por ejemplo, anticuerpos monoclonales antidiana no mielotóxicos).

- Pacientes con tratamientos onco-hematológicos no citotóxicos con neutropenia (40mg/día de prednisolona durante más de una semana) por cualquier motivo en los treinta días previos. o Tratamiento en los tres meses anteriores con fármacos inmunomoduladores no biológicos: metotrexato (>20 mg/semana o >15 mg/m^2 /sem, oral o subcutáneo), leflunomida, 6 mercaptopurina (>1,5 mg/kg/día) o azatioprina (>3 mg/kg/día), ciclosporina, micofenolato, tacrolimus (formas orales), sirolimus y everolimus en los tres meses previos.
- Tratamiento inmunosupresor con inmunomoduladores biológicos: Personas que han recibido en los tres meses anteriores (seis meses en caso de anti CD20) terapia específica con alguno de los fármacos de los siguientes grupos: o Anticuerpos monoclonales anti CD20 o Inhibidores de la proliferación de células B o Proteínas de fusión supresoras de linfocitos T o Inhibidores de la interleukina 1 (IL-1) o Anticuerpos monoclonales anti-CD52 o Moduladores del receptor de la esfingosina-1-fosfato o Inhibidores de la proteinquinasa o Inhibidores de la familia janus quinasa (JAK).

b) Personas no vacunadas* con >80 años.

c) Personas no vacunadas* con >65 años y con al menos un factor de riesgo para progresión**.

d) Personas vacunadas (>6 meses) con >65 años y con al menos un factor de riesgo para progresión**.

*Se consideran personas no vacunadas las personas que no han recibido la pauta de vacunación completa (incluidas las dosis de recuerdo) y no han padecido la enfermedad en los 3 últimos meses.

** Se consideran factores de riesgo de progresión:

- Enfermedad renal crónica: Pacientes con estadios de enfermedad renal crónica 3b, 4 ó 5 (Tasa de filtración glomerular inferior a 45 ml/min.
- Enfermedad hepática crónica: pacientes con una clasificación en la escala de Child-Pugh para gravedad de la enfermedad hepática de clase B o C (enfermedad hepática descompensada).
- Enfermedad neurológica crónica (Esclerosis múltiple, esclerosis lateral amiotrófica, miastenia gravis o enfermedad de Huntington).
- Enfermedades cardiovasculares, definidas como antecedentes de cualquiera de los siguientes: infarto de miocardio, accidente cerebrovascular (ACV), accidente isquémico transitorio (AIR), insuficiencia cardíaca, angina de pecho

con nitroglicerina prescrita, injertos de revascularización coronaria, intervención coronaria percutánea, endarterectomía carotídea y derivación aórtica.

- Enfermedad pulmonar crónica (EPOC de alto riesgo [FEV1 post-broncodilatación <50%, o disnea (escala modificada de disnea mMRC) de 2-4, o 2 o más exacerbaciones en el último año, o 1 ingreso]; asma con requerimiento de tratamiento diario).
- Diabetes con afectación de órgano diana.
- Obesidad (IMC≥35).
- Bajo peso (IMC≤18,5).

Tabla 5. Esquema de tratamiento en pacientes con factores de riesgo para progresión y enfermedad leve.

Enfermedad leve, pero con factores de riesgo de progresión para enfermedad grave.			
Grupo de riesgo 1 y serología negativa (IgG anti S) o bajo nivel de protección <260	Como primera opción: (≤ 5 días desde inicio síntomas): Nirmatrelvir/ritonavir (Paxlovid)	Ómicron (excepto variante BA.2) ≤ 5 días desde inicio síntomas: Sotrovimab (Xevudy)	No Ómicron ≤ 7 días desde inicio síntomas: Casirivimab/imdevimab (Ronapreve)
Grupos de riesgo 2, 3 y pacientes del grupo 1 con IgG anti S>260 BAU/mL o serología no disponible.	Como primera opción: (≤ 5 días desde inicio síntomas): Nirmatrelvir/ritonavir(Paxlovid)	Alternativamente: ≤ 7 días desde inicio síntomas: Remdesivir (Veklury) (3 días)	

[b] Se considera un nivel bajo de protección a infección por SARS-CoV-2, tal y como recoge el documento de la AEMPS, un título <260 BAU/ml según la OMS.

[d] Si no es posible el tratamiento con ninguna de las alternativas priorizadas, podrá valorarse molnupiravir en adultos con COVID-19 que no requieran oxígeno suplementario y ≤5 días desde inicio síntomas.

No se plantea tratamiento frente al COVID -19 si: Los sujetos son sintomáticos, pero sin factor de riesgo de progresión a enfermedad grave y; Ante infección asintomática por SARS-CoV-2.

2. *Pacientes hospitalizados con* FR de enfermedad grave*, pero sin oxigenoterapia*→ Se sugiere Remdesivir de la farmacéutica Gilead Sciences.
3. *Pacientes* hospitalizados por razones no COVID y con infección incidental y FR de desarrollar enfermedad grave→Se plantean terapias autorizadas con Nirmatrelvir-ritonavir (Paxlovid de Pfizer), terapia con anticuerpos monoclonales y remdesivir sí; tiene síntomas leves, inicio de los síntomas dentro de los 5 a 10 días anteriores y son de alto riesgo de progresión. Hay que tener en cuenta que el 1º día de síntomas se considera el día 0, el día siguiente se considera el día 1, etc.
4. *Pacientes hospitalizados con requerimiento* de oxígeno y/o enfermedad grave: El tratamiento depende del tipo de flujo de oxígeno que[70-71] reciben.
    a. Pacientes que reciben oxígeno a bajo flujo: Se recomienda dexametasona en dosis bajas y remdesivir. Si el flujo es muy bajo (1-2l/min) y el paciente está inmunodeprimido se desaconseja el uso de dexametasona. Para los pacientes que tiene signos inflamatorios elevados por los parámetros de laboratorio, y/o necesitan oxigeno creciente a pesar de este tratamiento se sugiere agregar baricitinib o tocilizumab si están dentro de las 96h posteriores a la hospitalización.
    b. Pacientes que reciben oxígeno de alto flujo o ventilación mecánica no invasiva (VMNI): Se recomienda dexametasona a dosis bajas y según evolución asociar agregar baricitinib o tocilizumab si están dentro de las 96h posteriores a la hospitalización.

Aunque también se recomienda remdesivir en este grupo, este fármaco antirretroviral se le da prioridad con los pacientes que necesitan bajo flujo.

Respecto a la elección de baricitinib o tocilizumab depende de la disponibilidad. NO hay estudios que comparen directamente a estos fármacos.

    c. Pacientes que requieres ventilación mecánica invasiva o ECMO: En estas situaciones se recomienda dexametasona a dosis bajas y asociar en caso de progresión baricitinib o tocilizumab .

Tratamientos específicos frente al COVID-19:

1. *Dexametasona y otros corticoides:* Se recomienda en pacientes graves con necesidad de oxígeno o soporte respiratorio. EL corticoide de elección es la dexametasona a dosis de 6mg/diarios durante 10 días o hasta el alta, pudiendo usarse otros como metilprednisolona, hidrocortisona y prednisona si no hay disponibilidad de la

primera. En el estudio RECOVERY se demostró como los glucocorticoides redujeron la mortalidad a 28 días en pacientes con COVID-19 grave en comparación al grupo que recibió placebo, sin asociar mayor riesgo de eventos adversos. En este metaanálisis no se observó beneficio del uso de corticoides en pacientes que no requerían oxigenoterapia ni soporte respiratorio.

2. *Inmunomoduladores adyuvantes*:
    a. Baricitinib (Olumiant de Eli Lilly) e inhibidores de JAK: Este fármaco es un inhibidor de la quinasa Janus (JAK) usado en la artritis reumatoidea (AR). Se cree que además tiene efectos antivirales interrumpiendo la entrada viral. Como ya hemos redactados en el apartado de esquema de tratamiento, el baricitinib se usa en pacientes con bajo flujo pero que tienen una tórpida evolución a pesar de la dexametasona y, en aquellos con alto flujo o soporte respiratorio. Nunca se utilizará si ya se ha usado otro fármaco inhibidor de la vía de la IL-6 (tocilizumab). Su dosis recomendada es de 4mg vía orales diarios durante un máximo de 14 días, reduciéndola en pacientes con filtrado glomerular menor de 15ml/min por 1,73m^2. Tampoco se recomienda su uso en pacientes con neutropenia o leucopenia. Su uso se basa en los ensayos donde demuestra reducir la mortalidad en pacientes con enfermedad grave a pesar del uso con corticoides, sin aumento aparente en el porcentaje de reacciones secundarias.
    b. Inhibidores de la vía IL-6 (por ejemplo, tocilizumab): La cascada inflamatoria que conlleva la enfermedad grave hace que el bloqueo de esta vía pueda prevenir la progresión. Se han propuesto así varios fármacos que bloquean los receptores de la IL-6 como el tocilizumab, Sarilumab y situximab.

       Tocilizumab (Actemra de Roche): Se recomienda agregarlo a pacientes con enfermedad grave o críticos que tienen marcadores inflamatorios elevados, pues la evidencia sugiere una reducción en mortalidad de estos. También se recomienda su uso en pacientes con oxígeno a bajo flujo que progresan con elevación de los marcadores inflamatorios. Se debe evitar este fármaco en personas con infecciones graves no controladas distintas al COVID-19, ante neutrofilia, elevación de transaminasas (aspartato aminotransferasa-AST->10 veces su límite permitido y, riesgo elevado de perforación intestinal.
    c. Remdesivir (Veklury de Gilead Sciences): Se ha evaluado en pacientes graves y no graves por COVID-19. Está indicado para el tratamiento de la enfermedad por COVID-19 grave en adultos y adolescentes (de 12 años y

mayores con un peso corporal de al menos 40 kg) que requieren oxígeno suplementario, pero no soporte ventilatorio (estudio SOLIDARITY) y, recientemente se ha aprobado su uso en adultos con enfermedad no grave, pero factores de riesgo. La dosis recomendada de remdesivir en pacientes de ≥ 12 años y que pesen al menos 40 kg es una dosis única de carga de 200 mg administrada mediante perfusión intravenosa y 100 mg administrados una vez al día mediante perfusión intravenosa a partir del día 2. La duración del tratamiento será de 3 días en pacientes no hospitalizados/no graves, y de 5-10 días en pacientes hospitalizados/graves. No se debe utilizar remdesivir en pacientes con una tasa de filtración glomerular (TFGe) <30ml/min, ALT≥5 veces el límite superior de la normalidad, embarazadas ni periodo de lactancia.

d. Terapias basadas en anticuerpos monoclonales: Opciones disponibles para pacientes ambulatorios sintomáticos con riesgo de progresión a enfermedad grave. Hay que destacar que, los anticuerpos monoclonales tienen una actividad variable contra las diferentes variantes del SARS-CoV-2, y la selección del agente debe considerar la susceptibilidad de las variantes circulantes. Además, requieren administración parenteral y deben administrarse precozmente; estos factores hacen que la administración sea complicada en entornos ambulatorios.

Bebtelovimab es el único anticuerpo monoclonal disponible en los Estados Unidos que es activo contra todos los sublinajes circulantes de Ómicron (incluidos BA.2, BA.4 y BA.5). Se administra como una dosis única de 175 mg iv tan pronto como sea posible después del diagnóstico y dentro de los siete días posteriores al inicio de los síntomas.

Otras terapias con anticuerpos monoclonales (es decir, sotrovimab- Xevudy de GlaxoSmithKline - [500 mg IV], casirivimab-imdevimab-Ronapreve de Roche- [600-600 mg como dosis única IV o subcutánea] y bamlanivimab-etesevimab de Eli Lilly [700-1400 mg como dosis única IV]) también han recibido autorización de uso de emergencia (EUA, por sus siglas en inglés) para el tratamiento de COVID-19 no grave en pacientes ambulatorios con riesgo de progresión, pero estos agentes **no** están autorizados ni son apropiados para su uso cuando el COVID-19 se debe a Ómicron y sus variantes no susceptibles.

e. Plasma de convalecientes: Consideramos que el plasma de convaleciente de título alto es una opción alternativa para la terapia específica de COVID-19

para pacientes ambulatorios sintomáticos con riesgo de progresión a enfermedad grave si nirmatrelvir-ritonavir (Paxlovid) , bebtelovimab o remdesivir (Veklury) no están disponibles o adecuado.

f. Nirmatrelvir-ritonavir (Paxlovid): Se trata de la primera opción en pacientes con enfermedad leve-moderada ambulatorios con riesgo de progresión. Se trata de una combinación de inhibidores de proteasa orales que bloquean la actividad de la proteasa SARS-CoV-2-3CL, una enzima necesaria para la replicación viral. La administración conjunta con ritonavir ralentiza el metabolismo del nirmatrelvir para que permanezca durante más tiempo y en concentraciones más altas. Debe iniciarse lo antes posible después del diagnóstico de COVID-19 y dentro de los cinco días posteriores al inicio de los síntomas. Para pacientes con función renal normal, la dosis es de 300 mg de nirmatrelvir (dos tabletas de 150 mg) con una tableta de 100 mg de ritonavir tomadas juntas por vía oral dos veces al día durante 5 días. Como precaución hay que tener en cuenta las reacciones cruzadas con múltiples fármacos que tiene.

g. Molnupiravir (Lagevrio de Merck): Es un análogo de nucleósido que inhibe la replicación del SARS-CoV-2. Lo consideramos una opción para pacientes ambulatorios con riesgo de progresión donde no pueden usarse otros fármacos de elección previa. La dosis es de 800 mg (cuatro cápsulas de 200 mg) por vía oral cada 12 horas durante cinco días. Debe iniciarse lo antes posible después del diagnóstico de COVID-19 y dentro de los cinco días posteriores al inicio de los síntomas. Como ventaja, no es necesario ajustar la dosis en función de la insuficiencia renal o hepática.

Aunque se aconsejaron diferentes fármacos antivirales e inmunomoduladores a lo largo de la pandemia COVID-19, se desaconseja su uso en la actualidad dada la ausencia de evidencia en los ensayos clínicos. Hablamos de: Hidroxicloroquina/cloroquina; Favipiravir; Interferones; Inhibidores de la IL-1 (Anakinra-kineret- de Amgen); Azitromicina; Lopinavir-ritonavir; Ivermectina; Vitamina D; corticoides inhalados; Colchicina.

Se aconseja continuar con los medicamentos crónicos que planteaban dudas (inhibidores de la ECA o bloqueantes de los receptores de la angiotensina (ARA), así como recibir profilaxis farmacológica en pacientes hospitalizados para prevenir la tromboembolia venosa.

Medicamentos de prevención.

La FDA ha emitido una autorización de uso de emergencia para el tixagevimab/cilgavimab (Evusheld de AstraZeneca), un medicamento que puede ayudar a protegerlo de contraer COVID-19. EVUSHELD contiene dos anticuerpos diferentes y se administra en forma de dos inyecciones intramusculares individuales consecutivas.

4.3 Condición post COVID-19.

4.3.1. Concepto de condición post COVID-19.

La batalla contra el COVID-19 no termina con el diagnóstico y tratamiento durante la fase aguda, sino que pueden desarrollarse secuelas y sintomatología persistente semanas o meses después, independientemente de la gravedad de éste[26] en un porcentaje de pacientes no despreciable.

En la actualidad, no existe un acuerdo en la definición ni en la cronología del período post-COVID-19. Por ello, se han usado múltiples términos como; COVID prolongado (long-haul COVID), síndrome post-COVID agudo (post-acute COVID-19 syndrome), secuelas post-agudas de COVID-19 (post-acute sequelae of COVID-19), trastornos post-COVID (post-COVID conditions), síndrome post-COVID-19 (post-COVID syndrome -SPC-) y, se han publicado diferentes definiciones al respecto. Ya existen códigos específicos ICD-10 (U09) e ICD-11 (RA02) para identificar todos estos términos[72].

La siguiente tabla es una representación de las diferentes definiciones publicadas de los conceptos post COVID.

Tabla 6. Diferentes definiciones disponibles de la condición post COVID-19 (CPP).

Fuente	Definición
Lancet	En la conferencia de la Academia China de Ciencias Médicas-Lancet celebrada el 23 de noviembre de 2020, Bin Cao presentó datos (en prensa en Lancet) sobre las consecuencias a largo plazo de la COVID-19 en pacientes en Wuhan, y alertó de que en algunos pacientes dados de alta las disfunciones y complicaciones podían persistir durante por lo menos seis meses. La denominada COVID persistente es una creciente preocupación de salud, y se requiere actuar inmediatamente para abordarla
NICE	COVID-19 agudo/ LONG COVID: COVID-19 sintomático subagudo o en curso/ sd crónico o post-covid-19.

	Sd post covid-19: Los signos y síntomas manifestados durante o después de una infección confirmada por la COVID-19 persisten durante >12 semanas y no pueden explicarse por un diagnóstico alternativo.
Scientific American	Personas con síntomas que persisten o se manifiestan después de la infección vírica inicial, pero se desconocen su duración y patogénesis
Royal Society	La ocurrencia de episodios persistentes o recurrentes de uno o más de los siguientes síntomas, dentro de x* semanas de la infección por el SARS-CoV-2 y que persisten durante y* semanas o más: fatiga intensa, capacidad de ejercicio reducida, dolor de pecho o pesadez, fiebre, palpitaciones, disfunción cognitiva, anosmia o ageusia, vértigo y tinnitus, cefalea, neuropatía periférica, sabor metálico o amargo, erupción cutánea, dolor o inflamación articular (3). * El periodo máximo entre el momento de contraer la infección (si se conoce) y la aparición de síntomas, y la duración mínima de los síntomas, deberían especificarse en la definición.
Nature	COVID-19 agudo y COVID-19 post-agudo. Post-aguda: síntomas persistentes y/o complicaciones posteriores >4 semanas.
CDC	Post-COVID conditions: Consecuencias en salud >4 semanas; Síntomas nuevos o continuos+ secuelas 2º a daños multiorgánicos + efectos de la propia enfermedad y hospitalización.
Wikipedia	Afección caracterizada por secuelas a largo plazo —que persisten tras el periodo habitual de convalecencia— de la enfermedad por el coronavirus 2019 (COVID-19)
OMS	Post-COVID conditions: personas con historial de infección presunta o confirmada, >3 m después de contraer la COVID-19, con síntomas que duran> 2m y que no pueden explicarse por un diagnóstico alternativo. Los síntomas también pueden fluctuar o reaparecer con el tiempo.

El 22 de enero de 2021 el instituto nacional de excelencia y asistencia sanitaria[73] (NICE por sus siglas en inglés) publicó una guía definiendo los conceptos de covid-19 agudo, covid-19 sintomático en curso o subagudo ("ongoing symptomatic COVID") y síndrome post-covid-19, según la duración de los síntomas. Utiliza el término de *Long COVID* por primera vez refiriéndose a:

1. COVID-19 sintomático subagudo o en curso, que incluye síntomas y anomalías presentes de 4 a 12 semanas después del COVID-19 agudo;
2. Síndrome crónico o post-COVID-19, que incluye síntomas y anomalías que persisten o están presentes tras 12 semanas del proceso aguda y no son atribuibles a diagnósticos alternativos.

El 22 de marzo 2021, Nature[74] describe el COVID-19 post agudo como una serie de síntomas persistentes y/o complicaciones más allá de las 4 semanas desde el proceso agudo. Distingue así el:

1. Covid-19 agudo, haciendo referencia a los síntomas que continúan hasta 4 semanas desde la infección aguda.
2. Covid-19 post agudo, incluyendo los síntomas que persisten más de 4 semanas desde la infección aguda o si aparecen complicaciones pasado este tiempo. Este último término engloba los pacientes con covid-19 persistente (long COVID) y con secuelas post-COVID-19.

El 16 de septiembre de 2021, la CDC[75] plantea la utilización genérica de "post COVID conditions" o condición post COVID (PCC) para las consecuencias en salud que persisten más de 4 semanas desde el proceso agudo. Además, describe las posibles alteraciones que podemos encontrar en la práctica clínica relacionados con esta condición:

1. Síntomas continuos o nuevos persistentes: Son extremadamente numerosos y variables, pudiendo presentarse superpuestos y, variando o a lo largo del tiempo. El cansancio es el síntoma más frecuente (presente en el 60-70%). La disnea, con frecuencia asociada con tos y dolor torácico inespecífico, es característica y prolongada en el tiempo. En el área neurocognitiva destaca la disminución de la capacidad de concentración *(brain fog)*, alteraciones de memoria, inestabilidad en la marcha, temblores, cefalea y persistencia de ageusia y anosmia. Los síntomas ansioso-depresivos, así como las alteraciones del sueño, son también muy frecuentes. Otras manifestaciones son la alopecia, artralgias, mialgias, taquicardia o alteraciones del ritmo gastrointestinal, aunque se han descrito más de 50 síntomas distintos [74,76-77].
2. Secuelas secundarias a daños orgánicos: Debidas fundamentalmente al daño funcional que sufrieron los diferentes órganos (pulmón, corazón, riñón, cerebro, hígado, piel, etc.) durante la fase aguda y las posibles alteraciones autoinmunes desencadenadas.

También se han descrito, sobre todo en niños, el síndrome inflamatorio multisistémico (MIS, por sus siglas en inglés) durante o inmediatamente después de una infección por COVID-19.

3. Efectos de la propia enfermedad y/u hospitalización: Como la debilidad severa, trastornos de estrés postraumático, síndrome de cuidados intensivos posteriores (PICS, por sus siglas en ingles), que se refiere a los efectos en la salud tras saber estado hospitalizado en la unidad de cuidados intensivos (UCI), etc.

Finalmente, el 16 de diciembre de 2021 la OMS[78] también establece el término de condición post COVID-19 para personas con un historial de infección probable o confirmada por el

SARS-CoV-2, con manifestaciones clínicas residuales 3 meses después del proceso agudo y que duran al menos 2 meses sin poder explicarlos por otros diagnósticos alternativos. La clínica varia en el tiempo, fluctuando, habiendo por tanto periodos de mejoría y de empeoramiento.

Otras enfermedades víricas sistémicas, como el virus del Epstein-Barr, virus influenza A(H7N9), la fiebre del Nilo Occidental, el virus del Ébola, el sarampión, y previos coronavirus (SARS-CoV y MERS-CoV)[79-81] se han asociado a secuelas post infecciosas a largo plazo en ausencia de infección activa. La información publicada tras las epidemias producidas por otros coronavirus SARS-CoV y MERS-CoV evidencia la sintomatología persistente con el empeoramiento de la calidad de vida y, el deterioro de la función pulmonar a largo plazo que incluso se ha descrito 15 años después[82-85].

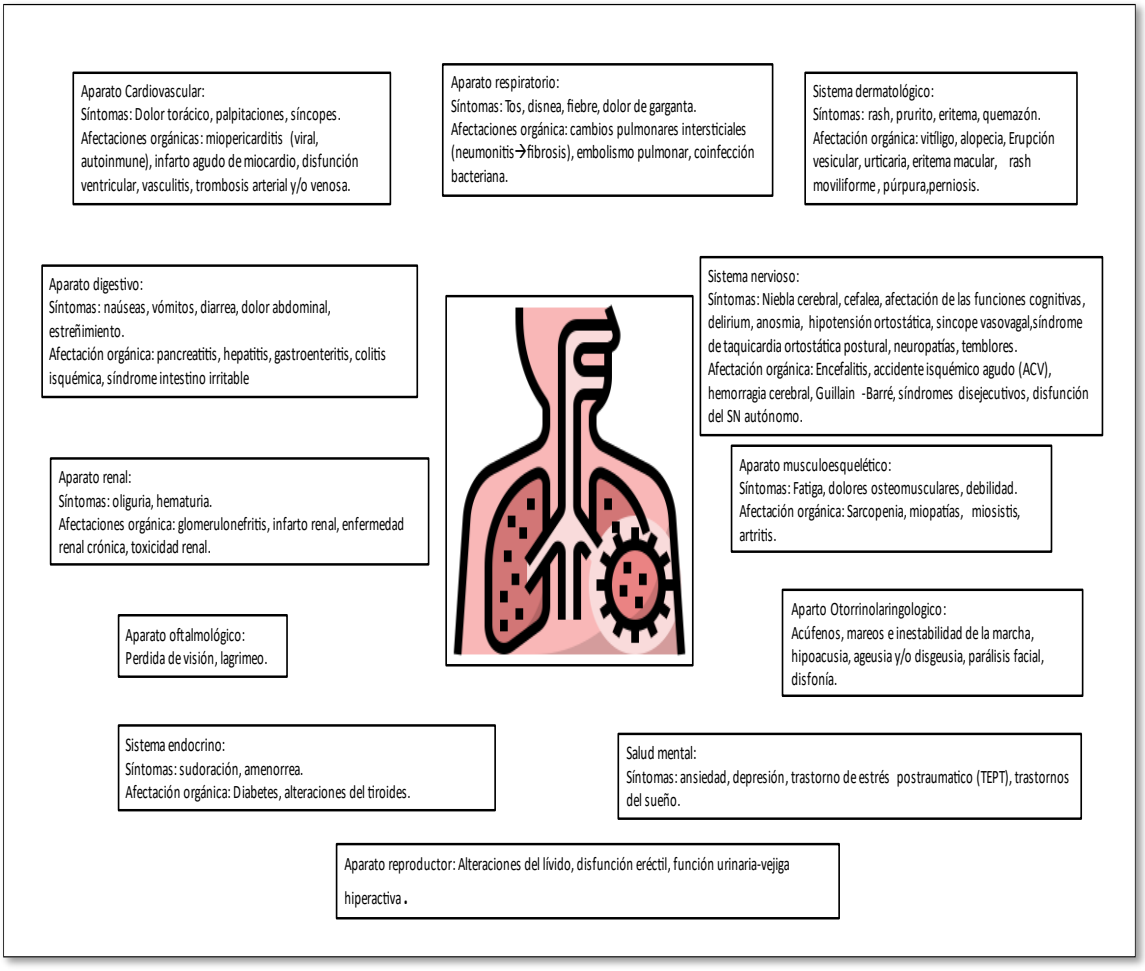

Figura 10. Tipos de condición post COVID-19 que podemos encontrar en la práctica clínica.

4.3.2. Etiología de la condición post COVID-19.

Se desconoce cuánto tiempo puede durar el período de recuperación y los motivos de la notable variación interindividual de las secuelas. También se desconocen las bases fisiopatológicas de estas secuelas y se barajan distintas teorías[86-87].

1. Estado de hiperinflamación crónica: Se observan niveles altos y alterados de inflamación en muchos pacientes durante la fase aguda. Se ha identificado la persistencia de una hiperrepuesta inmune citotóxica persistente quedando por aclararse los diferentes mecanismos de desregulación de la respuesta inmune, sea por exceso y/o por defecto. En el estudio publicado de Vibholm et al (2021)[88] se describía como tras 90 días, el 5,3% de los sujetos tenían PCR aún positivas. Si bien no hubo diferencias entre los niveles de anticuerpos contra SARS-CoV-2 entre los sujetos positivos y negativos para PCR, el grupo positivo mostró mayores niveles de células T CD8, lo que hacía sugerir que aquellos sujetos podrían albergar aún virus replicantes. Además, las alteraciones inmunitarias que provoca dicho virus pueden conllevar la reactivación de patógenos albergados previamente, infectando nuevos sitios del cuerpo y provocando síntomas crónicos. De ahí que se haya publicado que la CPP pueden ser una consecuencia de la reactivación del virus Epstein Barr (VEB)[89] inducida por la inflamación propia de la enfermedad en sí.
2. Persistencia del virus: Originando una infección crónica. Diferentes publicaciones describen la existencia del virus acantonado en el tubo digestivo, así como en la mucosa olfatoria. Se ha descrito el hallazgo de proteína viral hasta 15 meses después en pacientes con dicha condición.
3. Estado de hipercoagulabilidad producido por plaquetas y coágulos (estado tromboinflamatorio), responsable de tasas elevadas de complicaciones trombóticas observadas en pacientes con COVID-19 en dicho periodo.
4. Daño producido por el efecto de la autoinmunidad: Las células B también están involucradas en la autoinmunidad prolongada del COVID-19. La existencia de autoanticuerpos que actúan contra proteínas inmunomoduladoras (citoquinas, componentes del complemento, proteínas de la superficie celular) alteran la función inmunitaria con el deterioro del control virológico[90].En pacientes con CPP se han encontrado elevado número de autoanticuerpos. Y ciertas enfermedades autoinmunes, como el lupus y la artritis reumatoide, comúnmente causan fatiga y problemas digestivos.

5. Alteraciones del microbioma del huésped: Las alteraciones inmunitarias que conlleva el SARS-CoV-2 pueden producir desequilibrios de los ecosistemas microbianos y virales del cuerpo humano que conllevarían síntomas persistentes. Así, varios estudios sugieren que dicho virus puede provocar disbiosis, es decir, un disbalance del equilibrio microbiano de la flora normal dando lugar al crecimiento de patógenos oportunistas y al agotamiento de especies comensales.
6. Alteraciones del sistema nervioso: La disautonomía, que se refiere a una desregulación del sistema nervioso autónomo, afecta el flujo sanguíneo incluido el cerebral, por lo que puede causar fatiga, dolores de cabeza, confusión mental e intolerancia al ejercicio. Se objetiva un cuadro de hiperactivación noradrenérgica que podría ser secundario al desajuste del sistema nervioso autónomo.

En resumen, parece que múltiples mecanismos[90]pueden confluir en el mismo paciente, con el desarrollo de esta sintomatología tan amplia y variada.

4.3.3. Condición post COVID-19 en niños.

Los datos clínicos de estas alteraciones en los niños son limitados. La sintomatología persistente más frecuente es el dolor osteomuscular, cefalea, sensación de opresión o dolor torácico, palpitaciones, problemas respiratorios y trastornos del sueño. Respecto a factores de riesgo del desarrollo de estas alteraciones se han descrito la edad avanzada y las enfermedades alérgicas[91].

4.3.4. Prevalencia de la condición post COVID-19.

La CDC describe a 29 de septiembre de 2022 una tasa de 13.480.310 casos y 113.217 muertes en España[96]. Los estudios publicados sobre la prevalencia de la CPP hablan sobre porcentajes dispares, debido a que tienen en cuenta diferentes poblaciones (hospitalizados o no), diferentes momentos del periodo de convalecencia, sintomatología auto informada o recogida por encuestas rigurosas validadas, etc. No obstante, según la CDC a 1 de septiembre de 2022, la CPP estará presente en el 13.3% de los pacientes al mes de la infección aguda y, en más del 30% a los 6 meses entre los pacientes que fueron hospitalizados[75,92]. La OMS describe que entre un 10-20% de las personas lo desarrollarán.

Las estimaciones del Instituto de Métricas y Evaluación de la Salud (IHME, por sus siglas en inglés) de la Universidad de Washington en Seattle, indican que el 6,8% (1,3%-12,4%) de todas las infecciones SARS-CoV-2 y el 15,2% (2,8%-27,6%) de pacientes con infección sintomática desarrollan esta condición[93].

En la actualidad en España, el registro Regicovid-AP está trabajando para informar sobre dicha prevalencia[94]. Pero, ante la falta de un registro nacional de pacientes con dicha condición y, considerando que un 10% de supervivientes de COVID-19 desarrollan posteriormente dicha condición, puede estimarse que más de 1 millón de personas pueden desarrollarlo.

4.3.5. Factores de riesgo asociados a condición post COVID-19.

Respecto a los factores de riesgo que puedan influir en el desarrollo de dicha condición, se ha sugerido que la presencia de sexo femenino, tener más de 5 síntomas los primeros 7 días de la infección aguda y presencia de comorbilidades incrementa dicho riesgo[95]. Respecto a la gravedad de la infección aguda las series son contradictorias entre sí[96]. La edad no parece ser un factor de riesgo y, no está clara la asociación con la comorbilidad, que puede comportarse como un factor de confusión en la interpretación de los síntomas[97].

5. JUSTIFICACIÓN Y OBJETIVOS DEL ESTUDIO:

5.1. Justificación.

Otras enfermedades víricas sistémicas, como el VEB, la fiebre del Nilo Occidental, el virus del Ébola, el sarampión, así como previos coronavirus SARS-CoV y MERS-CoV se han asociado a secuelas post infecciosas a largo plazo en ausencia de infección activa. La literatura publicada evidencia la sintomatología persistente con el empeoramiento de la calidad de vida y, el deterioro de la función pulmonar a largo plazo que incluso se ha descrito 15 años después, por lo que también era esperado que se desarrollasen en los pacientes post-COVID-19. Esto justificó la primera de las publicaciones que forman parte de esta tesis donde profundizamos en la clínica persistente y en los daños estructurales que están presentes tras el COVID-19.

Además, entender que mecanismos subyacen en la PCC es fundamental para avanzar en las terapias dirigidas. Por ello, nos planteamos si la inflamación crónica de los distintos órganos puede ser una de las causas que expliquen dichas secuelas. En concreto, nos preguntamos si el PET puede ayudarnos en el seguimiento de los pacientes post-COVID, pues dicha técnica nos aporta información tanto morfológica como funcional. La PET-TC con [18F]- (FDG) ofrece un análisis cuantitativo del metabolismo glucémico y, por tanto, de la intensidad de la actividad inflamatoria. Las variables del valor de captación estandarizado (SUV), el volumen tumoral metabólico (MTV) y la glucólisis total de la lesión (TLG) podrían utilizarse para estimar la actividad inflamatoria de los pulmones u otros órganos. Esta hipótesis justificó la investigación realizada en la segunda publicación de esta tesis.

Por último, identificar qué factores de riesgo pueden influir en la evolución del COVID-19, así como en el desarrollo de la CPP es necesario para poder evitarlo. Según la literatura disponible hasta este momento, los factores de riesgo que pueden contribuir al desarrollo de la CPP pueden clasificarse (por niveles de evidencia) en; establecidos, probables y posibles. Nos planteamos así el papel de la proteína α1-antitripsina (AAT) y su déficit (DAAT) en las secuelas pulmonares, en concreto en el deterioro de la función pulmonar, sabiendo que su déficit aumenta el riesgo de desarrollar una variedad de enfermedades, como el enfisema pulmonar. El estudio de los diferentes FRs y el papel de la AAT son los objetivos del tercer trabajo publicado de esta tesis.

El presente trabajo nace como respuesta a la necesidad actual de investigar y profundizar en la PCC dada la relevancia de esta patología y el desconocimiento de la misma. Así, en este trabajo investigaremos sobre la sintomatología persistente, los daños orgánicos residuales, el papel del PET TC en el seguimiento y, las relaciones entre las distintas variables que pueden

predisponer al desarrollo de la PCC, consiguiendo de este modo, tener una visión lo más amplia y real de la situación en nuestro medio.

5.2. Objetivos.

El objetivo de este trabajo es estudiar la condición post COVID-19 de forma multidimensional.

Para ello, trabajaremos en los siguientes objetivos específicos:

Objetivo 1: Analizar de forma exhaustiva la CPP 6 meses después del proceso agudo y, la influencia que pueden tener en el desarrollo de esta (ya sea por sintomatología persistente y/o presencia de secuelas orgánicas variables) las variables de género, la gravedad de la enfermedad durante la fase aguda y, la presencia de comorbilidades respiratorias previas (Artículo 1 de la sección Resultados).

Objetivo 2: Evaluar la utilizad de la tomografía por emisión de positrones (PET/TC) con-[18F]-fluoro-2-dexosi-D-glucosa ([18F]-FDG) en el seguimiento a corto plazo de pacientes ingresados por neumonía COVID-19 y, explorar la asociación de dichos hallazgos con los marcadores de pronóstico clínicos (Artículo 2 de la sección Resultados).

Objetivo 3: Identificar las condiciones médicas subyacentes que pueden predisponer al deterioro de la función pulmonar 12 meses después de la infección por SARS-CoV-2 y, analizar el papel de la proteína alfa 1 antitripsina (AAT) en dichas alteraciones (Artículo 3 de la sección Resultados).

6. METODOLOGÍA:

Esta tesis está compuesta por los resultados obtenidos en tres estudios observacionales prospectivos llevados a cabo en población granadina del área Norte pertenecientes al HUVN.

Ambos estudios fueron aprobados por el Comité de ética de investigación de Granada y, ambos cumplen los criterios establecidos por la declaración de Helsinki de 1975 revisados en 2008 (Word medical association declaration of Helsinki; Ethical principles for medical research involving human subjects.2013; Shephard, 1976). Se han seguido las indicaciones STROBE (Strengthening the Reporting de Observational estudios in Epidemiology) durante la realización de estos.

6.1 Artículo 1: Asociaciones únicas y múltiples de las secuelas post-agudas del COVID-19: Estudio de cohorte prospectivo de 6 meses de duración.

Contexto y diseño.

Se trata de un estudio observacional de cohortes prospectivo realizado en el HUVN en Granada, España. Se programaron dos visitas de seguimiento en el período de reclutamiento de datos llevado a cabo desde mayo a octubre de 2020. EL estudio sigue las indicaciones STROBE, los requisitos de la declaración de Helsinki y la ley Orgánica 15/1999 de protección de datos de carácter personal. Después de la declaración de Helsinki, se obtuvieron los consentimientos informados por escrito de todos los pacientes y, el comité de ética local lo aprobó.

Muestra.

Un total de 372 pacientes, con 217 incluidos y 115 excluidos, fueron remitidos a la primera consulta de seguimiento 2 meses después del diagnóstico de COVID-19. De ellos, 207 completaron la segunda revisión a los 6 meses del proceso agudo, siendo excluidos del estudio los 10 restantes. El cronograma del estudio, el diagrama de flujo y los procedimientos que se llevaron a cabo en cada consulta de seguimiento se muestran el apartado resultados, artículo 1, sección metodología.

La población del estudio estuvo constituida por pacientes ≥14 años, con diagnóstico de infección confirmado por RT-PCR de las vías respiratorias superiores (hisopo nasofaríngeo y orofaríngeo) o del tractor respiratorio inferior (cultivo de esputo) y serología de anticuerpos (IgM e IgG) por ELISA, y, que aceptaron firmar el consentimiento informado. Los criterios de

exclusión fueron; los casos sospechosos, los pacientes inmunodeprimidos con limitación terapéutica por patología terminal y aquellos que se negaron a participar.

Variables.

Las características clínicas estudiadas en cada visita de seguimiento fueron: tos, tipo de tos (seca o productiva), disnea, fatiga, debilidad muscular, afectación musculoesquelética, dolor torácico, palpitaciones, síndrome febril, alteraciones gastrointestinales, desarrollo de hipertensión arterial post COVID-19, síntomas de la esfera otorrinolaringológicos, oftalmológicos, manifestaciones neurológicas, alteraciones cutáneas, afectación del apetito sexual y, desarrollo de eventos tromboembólicos. Además, en cada consulta se incluyó variables de la salud mental, la exploración física e índices de laboratorio.

En todos los pacientes se realizó una radiografía de tórax en proyección postero-anterior y lateral en la primera consulta de seguimiento. Si había alteraciones radiológicas o continuaba con clínica de disnea se ampliaba el estudio con un TCAR de tórax para la segunda consulta médica. Los TCAR fueron evaluados por especialistas radiólogos y neumólogos describiendo los hallazgos según las recomendaciones de la sociedad española de radiología médica (SERAM), y la nomenclatura estándar internacional definida por la sociedad de Fleischer [98-99].

Cada prueba de imagen se analizó según: la densidad (patrón en vidrio deslustrado, consolidación alveolar, mixto -alveolo intersticial), la localización (central, periférica o difusa), la distribución (uni o bilateral), presencia de patrón reticular (subpleural o interlobulillar), porcentaje de extensión pulmonar afectada <20%, 20-50% y >50% según los campos pulmonares involucrados.

En la segunda consulta de seguimiento, cada paciente se sometió a unas pruebas funcionales completas con espirometría forzada, pletismografía y difusión pulmonar, además de un test de la marcha de los 6 minutos (TM6M). Los valores de referencia de dichas variables funcionales fueron tomados de la sociedad torácica americana y la normativa española[100-101], incluyendo el volumen espiratorio forzado en el primer segundo (FEV1), capacidad vital forzada (FVC), la relación FEV1/FVC, el volumen residual (VR), la capacidad pulmonar total (TLC), la difusión pulmonar de monóxido de carbono (DLCO) y la constante de difusión (KCO).

Análisis estadístico.

El análisis descriptivo se realizó utilizando el número y porcentaje, la mediana y el rango intercuartílico, combinando gráficas de cajas y de densidad, es decir, gráficos de violín[102], para variables categóricas y continuas, respectivamente[103]. Las discrepancias en las distribuciones analizados de los pacientes se presentan como diferencias con intervalos de

confianza (IC) del 95%. Se uso la prueba de U de Mann-Whitney para datos continuos de distribución no normal y, la X2 o el test de Fisher para comparar las características clínicas, el examen físico, la salud mental y, los índices de laboratorio según sexo recogidos en ambas consultas.

Se realizó un análisis bi y multivariado, calculando la odds ratios (OR) y los IC al 95% para explorar la asociación con las siguientes características; existencia de comorbilidades respiratorias previas, pacientes con necesidad de hospitalización durante el proceso agudo y, hallazgos anormales en el TC de tórax. El grado de asociación entre condición post COVID con el resto de las variables se define de acuerdo con el valor de OR (robusto o no). El programa para el procesamiento y análisis de los datos fue Python 3.7[104], considerando un valor de p inferior a α=0,005 estadísticamente significativo.

6.2 Artículo 2: PET/CT con [18F]FDG en las complicaciones a corto plazo de COVID-19: Marcadores metabólicos de inflamación persistente y deterioro de la función respiratoria.

Contexto y diseño.

Se trata de un estudio observacional de cohortes prospectivo realizado en el HUVN en Granada, España. Concretamente se trata de un estudio de pilotaje sobre una cohorte naturalística de una serie de casos que se siguen de forma prospectiva desde el momento del ingreso (noviembre 2020) hasta completar el seguimiento (marzo 2021) en nuestro centro hospitalario con dos visitas de seguimiento. EL estudio sigue las indicaciones STROBE, los requisitos de la declaración de Helsinki y la ley Orgánica 15/1999 de protección de datos de carácter personal. Después de la declaración de Helsinki, se obtuvieron los consentimientos informados por escrito de todos los pacientes y, el comité de ética local lo aprobó.

Muestra.

Se incluyeron 20 pacientes que fueron hospitalizados durante la 3º ola pandémica de España y que completan un seguimiento desde noviembre 2020 hasta marzo 2021. En la 1º revisión médica a los 2 meses del alta hospitalaria se realizó un PET TC y, en la 2º revisión médica a los 3 meses de dicha alta se hicieron unas pruebas de función respiratoria y TM6M. Los criterios de inclusión fueron; casos confirmados de COVID-19 de acuerdo con las directrices de la OMS y, hallazgos de neumonía COVID-19 durante el ingreso ya fuese en la radiografía o TCAR de tórax. Los criterios de exclusión fueron edad menor de 18 años, ausencia de confirmación microbiológica de la infección por COVID-19, antecedentes o presencia de fibrosis pulmonar, infección activa o no controlada por COVID-19 en el momento de la

realización del PET-TC, antecedentes o sospecha de enfermedad oncológica, embarazo o incapacidad para firmar el consentimiento informado.

Variables.

De todos los pacientes se recogieron datos al ingreso hospitalario sobre comorbilidades basales, clínica a debut, pruebas analíticas y, pruebas radiológicas. A las 48-72h del ingreso y en el examen de seguimiento del PET TC se repitieron las pruebas analíticas que incluían hemograma completo, bioquímica sanguínea estándar, reactantes de fase aguda, estado de coagulación y cociente de neutrófilos/linfocitos (NLR).

Durante el ingreso hospitalario, todos los pacientes fueron sometidos a una radiografía de tórax en proyección postero-anterior y lateral y/o un TCAR de tórax, informados por radiólogos especialistas según las recomendaciones de la SERAM, y la nomenclatura estándar internacional definida por la sociedad de Fleischer 102-103. Se definieron las características en cuanto a; la densidad (patrón en vidrio deslustrado, consolidación alveolar, mixto), la localización (central, periférica o difusa), la distribución (uni o bilateral) y, la extensión (uni o multilobar).

La prueba del PET TAC con [^{18}F]FDG se realizó a los 2 meses del alta médica. El protocolo de prueba se basó en las recomendaciones internacionales[105].Las imágenes fueron analizadas por dos médicos de medicina nuclear con experiencia en la interpretación de imágenes cardiotorácicas de forma independiente. Estaban cegados a los datos biológicos y clínicos de los pacientes y, las discrepancias en las interpretaciones se resolvieron por consenso de un tercer médico experto en dicha especialidad. Las regiones de interés (ROI) se dibujaron en imágenes de TC pulmonar alrededor de áreas con pérdida evidente de aireación y áreas adyacentes de apariencia normal. Lo mismo se hizo con imágenes de TC de ganglios linfáticos mediastínicos. Se calculó la captación patológica de 18F-FDG para cada ROI, determinando el SUV máximo, pico y mínimo (SUV max, SUV pico y SUV min, respectivamente) normalizados por el peso corporal y, la masa corporal magra (SUL); volumen tumoral metabólico (MTS; volúmenes de pixeles en el ROI con SUV max >40%) y, glucolisis total de la lesión (TLG; MTV multiplicado por SUV medio).

Las pruebas de función respiratoria se realizaron a los 3 meses del alta hospitalaria, con espirometría forzada, pletismografía, difusión pulmonar y el TM6M. Los valores de referencia de dichas variables funcionales fueron tomados de la sociedad torácica americana y la normativa española[100-101], incluyendo el FEV1, la FVC, la relación FEV1/FVC, TLC, DLCO y KCO.

Análisis estadístico.

Todas las mediciones para cada participante fueron realizadas independientemente por dos médicos de medicina nuclear, considerando el valor medio en los análisis estadísticos. Se calcularon número absolutos y porcentajes para las variables categóricas y medias con desviación estándar (DE) para las variables continuas. Para las comparaciones de datos cuantitativos entre los grupos PET positivo y negativo, se aplicó la prueba de T de student cuando la distribución era normal y la prueba de U de Mann-Whitney cuando no lo era. Las asociaciones con variables categóricas se evaluaron mediante la construcción de tablas de contingencia, aplicando la prueba de chi-cuadrado para comparaciones individuales y la prueba exacta de Fisher para comparaciones múltiples. Los resultados volumétricos de [^{18}F]FDG se correlacionaron con los resultados de las pruebas de laboratorio y los parámetros de la función respiratoria mediante el uso del coeficiente de correlación de rango de Spearman. Para los análisis estadísticos se utilizó el programa SPSS IBM versión 15 y el software R para procesar las imágenes del PET TC. Una $p \leq 0,05$ fue considera significativa en todas las pruebas.

6.3. Artículo 3: Niveles anormales de Alfa-1 antitripsina y otros factores de riesgo asociados al deterioro de la función pulmonar a los 6 y 12 meses de la hospitalización por COVID-19: estudio de cohortes.

Contexto y diseño.

Estudio de cohortes observacional y prospectivo, en el que se han incluido pacientes hospitalizados por COVID-19 durante los meses de febrero a mayo 2020, que fueron remitidos para su seguimiento a una consulta post-COVID de Neumología del HUVN, Granada, España. El periodo de seguimiento se realizó durante un año tras la infección aguda (mayo 2020-mayo 2021). Todos los pacientes se incluyeron de forma consecutiva y se programaron 3 visitas. La primera fue 2 meses después del alta hospitalaria. En ella se recogieron los antecedentes personales, las características del ingreso hospitalario, los parámetros basales, los datos físicos y, se realizó radiografía de tórax. La segunda revisión fue 6 meses tras el alta hospitalaria y se realizaron PFR y TM6M. La última revisión fue a los 12 meses del alta hospitalaria con analítica de control, determinación de AAT junto con el genotipo de dicha proteína, así como realización de nuevas PFR para los que tenían algún tipo de alteración funcional en las previas. EL estudio sigue las indicaciones STROBE, los requisitos de la declaración de Helsinki y la ley Orgánica 15/1999 de protección de datos de

carácter personal. Después de la declaración de Helsinki, se obtuvieron los consentimientos informados por escrito de todos los pacientes y, el comité de ética local lo aprobó.

Muestra.

Un total de 182 pacientes hospitalizados por infección por SARS-CoV-2 fueron derivados a las consultas post-COVID de Neumología para revisión. El estudio de seguimiento se realizó hasta completar un año de la infección aguda, acudiendo 168 pacientes a la revisión a los 6 meses y, 157 a la revisión a los 12 meses. Los criterios de inclusión fueron: pacientes de edad superior a 18 años, con diagnóstico de infección confirmado según las recomendaciones internacionales hospitalizados por neumonía covid-19; y, que firmaron el consentimiento informado. Los criterios de exclusión fueron: pacientes sin confirmación microbiológica de la infección por coronavirus y, aquellos que se negaron a participar o firmar el consentimiento informado, con proceso inmunosupresor subyacente o limitación de esfuerzo terapéutico.

Variables.

En todos los pacientes se recogieron las siguientes características, distinguiendo dos categorías según estuvieran relacionadas con el ingreso hospitalario o seguimiento desde consulta.

-*Durante el ingreso hospitalario:*

Se recogieron datos sobre las variables demográficas, los antecedentes médicos y las características del ingreso, describiéndose los parámetros de laboratorio relacionados con la gravedad o mortalidad de la enfermedad[106].

En dicho ingreso se les realizó un estudio de imagen mediante radiografía de tórax en proyección posterior-anterior y lateral en el momento del diagnóstico informada por radiólogos especialistas según las recomendaciones de la SERAM, la nomenclatura estándar internacional definida por el glosario de la Sociedad Fleischner y las publicaciones existentes hasta el momento de su realización [98-99]. De cada prueba de imagen realizada se describían las características en cuanto a la densidad (alveolar, en vidrio deslustrado o mixta), la distribución (central, periférica o difusa), la localización (unilateral o bilateral) y, la extensión (uni o multilobar).

-*Durante el seguimiento:*

Se solicitó para la revisión de los 6 meses PFR con medidas espirométricas, volúmenes pulmonares y capacidad de difusión de monóxido de carbono junto con el TM6M. La exploración funcional se realizó con personal de experiencia con los equipos de

MasterScreen Body, de marca Jaeger, Alemania. Los valores de referencia y criterios de aceptabilidad se basaron en la normativa europea y española[100-101]. En la revisión de los 12 meses se solicitaron nuevas PFR sólo a los pacientes con algún tipo de alteración funcional detectada en la revisión previa y, la determinación de la concentración plasmática de AAT y de la proteína C reactiva a todos los pacientes. Se realizó en el mismo acto clínico el genotipo de dicha proteína mediante frotis bucal del laboratorio Progenika (PAGT). El PAGT utiliza el hisopo bucal OCR100 autorizado por la FDA y aprobado por la comisión europea (CE). La prueba permite el análisis simultáneo de hasta 384 muestras por lote y es capaz de identificar las 14 variantes de deficiencia más frecuentes del gen SERPINA1. Esta prueba se basa en la amplificación mediante reacción en cadena de la polimerasa de ADN genómico y en la posterior hibridación con sondas alelo específicas, utilizando la tecnología xMAP de Luminex. Los datos obtenidos se procesan con el software PAGT para obtener el informe final. Este algoritmo de software convierte los genotipos de las variantes alélicas en alelos asociados. Cuando no se encontró ninguno de los 14 alelos estudiados el resultado se anotó como negativo y se interpretó como un alelo M, ya que la ausencia de cualquiera de estos 14 alelos sugiere con más de un 99% de probabilidad que el genotipo corresponde a PI*M.

El laboratorio del servicio clínico de Progenika procedió a realizar la secuenciación del gen SERPINA1 si no encontraba ninguna de las 14 mutaciones y el nivel sérico de AAT era <60 mg/dl, o por petición del del clínico responsable. La secuenciación completa del gen SERPINA1 (NM 001127701.1), se realizó mediante secuenciación de última generación-NGS (MiSeq Illumina) de los de los 7 exones del gen. Las secuencias obtenidas se analizaron utilizando el software CLC Genomics Workbench (Qiagen) para identificar las variantes presentes en la muestra. Sólo se incluyeron en el estudio las variantes que podían explicar la patogenicidad (variantes validadas en la literatura o que podían afectar a la a la actividad o expresión proteica).

Análisis estadístico.

En el análisis descriptivo se emplearon medidas de tendencia central (media) y dispersión (desviación estándar) para las variables continuas, y la distribución de frecuencias absolutas y relativas para variables categóricas. Para la comparación de datos cuantitativos entre los 2 grupos de las variables PFR alteradas a los 12 meses y AAT1 alteradas se utilizó la prueba de la t de Student para datos independientes paramétricos y la U de Mann-Whitney para los datos no paramétricos, así como el análisis de la varianza (ANOVA) o la prueba de Kruskal-Wallis, para estudiar la asociación entre variables cuantitativas y cualitativas con más de 2 categorías, aplicando la corrección de Bonferroni para comparaciones múltiples. La

asociación entre las variables categóricas se evaluó por medio de tablas de contingencia aplicando la prueba de chi cuadrado para comparaciones individuales o la prueba exacta de Fisher para comparaciones múltiples. Para estudiar la asociación entre las distintas variables relacionadas con las comorbilidades basales, características demográficas, clínicas, físicas y pruebas de laboratorio con la recuperación respiratoria funcional a los 12 meses de la hospitalización se utilizó el análisis de regresión logística binaria univariante, Finalmente, se ajustó un modelo multivariante a partir de aquellas variables que mostraron asociación significativa en el análisis univariante. Los datos se procesaron para su análisis mediante el software IBM SPSS Versión 25.0 (IBM Corp, Armonk, Nueva York) y/o software de computación matemática R. Un valor de $p < 0,05$ se consideró estadísticamente significativo.

7. RESULTADOS:

7.1 Artículo 1. Evidencias de calidad.

ON THE SINGLE AND MULTIPLE ASSOCIATIONS OF COVID-19 POST-ACUTE SEQUELAE: 6-MONTH PROSPECTIVE COHORT STUDY.

(Asociaciones únicas y múltiples de las secuelas post-agudas del COVID-19: Estudio de cohorte prospectivo de 6 meses de duración)

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On the single and multiple associations of COVID-19 post-acute sequelae: 6-month prospective cohort study.

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Abstract:

Medical research is progressing to clarify the full spectrum of sub-acute and long-term efects of the post-COVID-19 syndrome. However, most manuscripts published to date only analyze the efects of post-COVID-19 in patients discharged from hospital, which may induce signifcant bias. Here, we propose a pioneering study to analyze the single and multiple associations between post-COVID-19 characteristics with up to 6-months of follow-up in hospitalized and non-hospitalized COVID-19 patients. The cohort study was conducted from May to October 2020 at the University Hospital Virgen de la Nieves, the leading hospital assigned for patients with COVID-19 in Granada, Spain. A total of 372 and 217 patients—with

217 and 207 included in the first and second follow-up visits—were referred 2 and 6 months after diagnosing COVID-19, respectively. We find out that post-COVID-19 clinical and mental health impairment symptoms are correlated with patient gender. Logistic adjustments showed strong statistically robust single and multiple associations of demographic, clinical, mental health, X-ray, laboratory indices, and pulmonary function variables. The functional lung tests are good predictors of chest CT imaging abnormalities in elderly patients. Bilateral lung involvement, subpleural reticulum, ground-glass opacity, peripheral lung lesions, and bronchiectasis were the most common fundings of the high-resolution computed tomography images. Non hospitalized patients suffered more severe thromboembolic events and fatigue than those hospitalized.

Introduction

Long-term effects on multiple organ systems, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—pathogen of coronavirus disease 2019 (COVID-19)—is one of the current problems faced by patients after passing the disease[2,3].Preliminary studies report persisting symptoms of SARS-CoV-2, such as fatigue, dyspnea, cognitive deficit, arthralgia, impaired lung functions, and abnormal chest images[4–10]. Similar persistent symptoms were reported in patients from previous coronavirus infections—including the 2003 SARS epidemic and the 2012 Middle East Respiratory Syndrome (MERS)[11–16]—reinforcing concerns about post-COVID-19 syndrome (PCS)[17] (see definition of PCS in materials and methods). The work of Ongsobre et al. clearly shows the persistent and prolonged effects of lung function impairment one year after acquiring severe acute respiratory syndrome (SARS1)[15]. In addition, Liu et al. point out that deterioration of pulmonary functions and quality of life may occur up to 3 years after acute infection[18].

Due to the limited capacity of the hospitals, only a tiny fraction of people with COVID-19 are admitted to hospitals[19–22]. However, most manuscripts published—to date—only consider hospitalized patients for cohort studies, which may induce significant bias[23]. Current 12-, 6- and 3-month follow-up studies focus on persistent clinical, psychological, pulmonary function, physical problems, and chest CT imaging only for the discharged patients[8,10,24–29]. No study has yet reported the scope of post-acute sequelae of COVID-19 in a singular or multiple manners, including the non-hospitalized patients. Also, the association between pre-existing respiratory diseases and PCS is still unknown.

Our goal is to analyze the degree of single and multiple associations between clinical characteristics, demographic features, mental health, and pulmonary function test linked to

PCS—of the first variant of SARS-CoV-2—in patients with/without previous respiratory diseases, hospitalized or not, and the abnormalities of chest CT images.

Materials and methods

Definiton of post-COVID-19 syndrome (PCS)

Signs and symptoms that develop during or after an infection consistent with COVID-19, present for more than 12 weeks, and are not attributable to alternative diagnoses—following the guideline of the National Institute for Health and Care Excellence (NICE), the Scottish Intercollegiate Guidelines Network (SIGN), and the Royal College of General Practitioners (RCGP)17.

Study population

The prospective cohort study was conducted at the University Hospital Virgen de la Nieves, one of the hospitals assigned for patients with COVID-19 in Granada, Spain. Two visits were scheduled in the follow-up period from May to October 2020. A total of 372 patients—with 217 included and 115 excluded—were referred 2-months after diagnosis of COVID-19 for the first follow-up consultation (FFuC). The 217 patients included in the FFuC were referred to the second follow-up consultation (SFuC) at 6-months after initial diagnosis—with 207 included and 10 excluded. For COVID-19 detection, was used the RT-PCR from the upper respiratory tract (nasopharyngeal and oropharyngeal swab) or lower respiratory tract (sputum collection) and antibody serology (IgM and IgG) by ELISA. The study timeline, flowchart, and follow-up consultation procedures are shown in Fig. 1. The study was conducted following the requirements of the Declaration of Helsinki and the Spanish Data Protection Act of 15/1999. Following the Declaration of Helsinki, written informed consent was obtained from all patients, and local ethics committees approved the study.

Figure 1: Timeline and flowchart illustrating the series of events in chronological order with the actions and tests conducted after the first case detected at the University Hospital Virgen de las Nieves. Starting with the first Covid-19 test results, the number of patients referred to post Covid-19 consultations, the details of the first and second consultations, and data analysis.

The study population consisted of patients aged > 14 years, diagnosis of infection confirmed according to international recommendations and signing the informed consent. The exclusion criteria were: suspected cases of SARS-CoV-2, immunosuppressed patients with therapeutic limitation due to terminal pathology, and those who refused to participate (see Fig. 1). The age, sex, BMI, toxic habits, profession, family members with confirmed or suspected COVID-19, and need or not of hospital admission were considered. See Table 2, which summarizes the characteristics of the patients included in the study.

Data collected in follow-up consultations

The clinical features studied at each follow-up visit were; dry and wet cough, dyspnea, fatigue, muscle weakness, musculoskeletal involvement, chest pain, palpitations, fever, sweating, intestinal disorders, post-COVID-19 arterial hypertension, otorhinolaryngologic symptoms, ocular symptoms, neurological manifestations, decreased sexual appetite, cutaneous manifestations and other expressed symptoms—thromboembolic events. Also, each consultation included mental abnormalities, physical examination, and laboratory indices (see Tables 1, 2).

TABLE 1. COMPARATIVE TABLE OF THE LABORATORY REFERENCE RANGES CONSIDERED NORMAL IN THE ADULT POPULATION ACCORDING TO THE INTERNATIONAL SYSTEM AND THE PROVINCIAL AREA OF GRANADA.

	Reference ranges	
Test in the study population (units)	**Laboratory-UH-VN**	**References** [1-3]
Biochemical		
Glucosa (mmol/L)	4·16-6·38	3·9-6·1
Total bilirubin (µmol/l)	5·13-20·52	0-26
Creatinine (µmol/L)	59·23 – 106·08	57-111
Alanine aminotransferase (ALT, SGPT) (U/L)	7-34	10-40
Aspartate aminotransaminase (AST, SGOT) U/L	10-35	10-40
Lactato deshidrogenasa (LDH), U/L	0 - 247	0-245
Serum ferritin (ng/mL)	10 - 120	21-274·66
Total protein (g/dL)	6·6 – 8·3	6·5-8·5
Hematologic		
Haemoglobin (g/dL)	12 - 15·6	13-17·5
Leukocytes (10^9 L)	3·9 – 10·2	3·5-9·5
Neutrophils (%)	42 - 77	50 -70
Lymphocytes (%)	20 - 44	30- 45
Lymphocytes (x10^9 L)	1·1 – 4·5	1·1-3·2

Platelets (x10^9 L)	130 - 370	125-359
Coagulation function		
D-dimer **(**µg/L)	0 – 0·5	0-1

Laboratory reference ranges and characteristics of the patients included in the study.

TABLE 2. SUMMARY OF THE CHARACTERISTICS OF THE PATIENTS INCLUDED IN THE STUDY.
CHARACTERISTICS (N=217**)**
Age (years), median (IQR)
Gender, N (%):
Male
Female
BMI (kg/m2)
Smoking history, N° (%):
Cumulative tobacco burden index (ICAT), N° (%):
Current
Former
Nonsmoker
Healthcare professional N° (%):
Need or not of hospital admission N° (%):
FIRST FOLLOW-UP CONSULTATIONS (N=217**)**
Clinical Features
Asymptomatic, N° (%)
Any one of the following symptoms
Dry cough, N° (%)
Wet cough, N° (%)
Dyspnea, N° (%)

Fatigue, N° (%)
Muscle weakness,N° (%)
Musculoskeletal involvement (arthralgia or myalgia), N° (%)
Chest pain,N° (%)
Palpitations,N° (%)
Fever (temperature ≥37·3°C),N° (%)
Sweating, N° (%)
Intestinal disorders, N° (%)
Nausea, N° (%)
Vomiting, N° (%)
Diarrhoea, N° (%)
Post covid arterial hypertension, N° (%)
Otorhinolaryngologic symptoms, N° (%)
Rhinorrhea, N° (%)
Sore throat or difficult to swallow, N° (%)
Tinnitus, N° (%)
Nasal dryness, N° (%)
Dysphonia, N° (%)
Hearing los, N° (%)
Loss or disturbance of taste or smell, N° (%)
Dizziness and gait instability, N° (%)
Ocular symtoms, N° (%)
Epiphora, N° (%)
Reduced visual acuity, N° (%)
Neurological Manifestations, N° (%)
Polyneuro/myopathy N° (%)

Headaches, N° (%)
Cognitive deficits, N° (%)
Erectile dysfunction, N° (%)
Decreased sexual apetite,N° (%)
Cutaneous manifestations, N° (%)
Skin rash, N° (%)
Rash or urticarial skin eruptions and other expressed symptoms, N° (%)
Hair loss, N° (%)
Thromboembolic events, N° (%)
Types thromboembolic events
Mental Health
Emotional affectation, N° (%)
Depression, N° (%)
Sleep disturbance, N° (%)
Physical Examination
Lung auscultation N° (%)
Crackles N° (%)
Oxygen saturation by pulse oximetry (Sat02), N° (%)
Nail clubbing, N° (%)
Abnormal finding on X-ray, N° (%)
Laboratory finding (Table 1)
SECOND FOLLOW-UP CONSULTATIONS (N=207)
Clinical features, mental health, physical examination, X-ray, and laboratory tests as in the FFuC.
Pre-existing respiratory disease (PRD), N° (%)
Pulmonary function
FVC <80%, % of predicted

FEV1 <80%, % of predicted
FEV1/FVC <70%
TLC <80%, % of predicted
VR <65%, % of predicted
DLCO <80%, % of predicted
KCO<80%, % of predicted
6MWT
Distance-meters, median (IQR)
Oxygen saturation, median (IQR)
Initial, Final, and Average
Chest CT
Density
Mixed pattern, Nº (%)
Consolidation, Nº (%)
Ground-glass, Nº (%)
Location
Peripheral,Nº (%)
Central,Nº (%)
Mixed,Nº (%)
Subpleural reticular pattern
Interlocular septal thickening, Nº (%)
Thickening of the adjacent pleura, Nº (%)
Lung involvement
Unilateral,Nº (%)
Bilateral,Nº (%)
Bronchiolectasis,Nº (%)

Others findings of CT, Nº (%)

In all patients, a chest radiograph was performed in posterior-anterior and lateral projection at the FFuC. Then, a complementary study with HRCT was requested if there were any abnormal findings on the X-ray. HRCTs were evaluated by radiologist specialists and one pulmonologist and reported according to the Spanish Society of Medical Radiology (SERAM) recommendations, the international standard nomenclature defined by the Fleischner Society glossary and existing publications until now. Each imaging test was analyzed considering: density (ground glass, consolidation or mixed), lung involvement (unilateral or bilateral), location (central, peripheral or mixed), presence of reticular pattern or interstitial lesions of pulmonary parenchymal (subpleural or interlobular), and the percentage of lung extension involved < 20%, 20–50% and > 50%—according to the lung fields involved (see Table 2.

At the SFuC, each patient underwent forced spirometry, lung volume, diffuse capacity, and the 6MWT. The functional exploration was carried out with experienced personnel with the equipment of MasterScreen Body, brand Jaeger, Germany. According to American Thoracic Society and Spanish regulations, the reference values for the Mediterranean population and acceptability criteria. Pulmonary parameters included FEV1, FVC, the FEV1/FVC ratio, RV, TLC, DLCO, and KCO.

Statistical analysis

Descriptive analysis was carried out using number (%), median, and its interquartile range (IQR)—combining box plots and density plots, i.e., violin plots—for categorical and continuous variables, respectively[40]. Discrepancies in the patient characteristic distributions by sub-groups of outcomes are presented as differences with 95% confidence intervals (CIs). The Mann–Whitney U test—for nonnormal distributed continuous data, $\chi 2$ test, or Fisher's exact was used to compare clinical features, physical examination, mental health, and laboratory indices between males and females at the first and second follow-up consultations.

Bivariate and multivariate analysis—using the maximum likelihood estimation to obtain the coefficients and the of Hosmer–Lemeshow goodness-of-fit test for the mode—was carried out to compute the odds ratios (ORs) and 95% CIs to explore the association with the following features: at least one pre-existing respiratory disease, hospitalized patients, and an

abnormal chest CT finding41. The degree of association of the PCS is defined according to the OR value (robust or not). Data cleaning and analysis using logistic regression models were implemented in Python 3.7. The tests were two-sided, and a p value less than $\alpha = 0.05$ was considered statistically significant.

Results

Of the 217 patients—of which 116 (53.5%) were male—with SARS-CoV-2 was examined, including hospitalized and not hospitalized patients. These patients were monitored from May to October 2020. The follow-up study from May to October 2020 was divided into two follow-up consultations. The FFuC was carried out two months after the diagnosis of infection—from May to mid-July 2020—and the SFuC six months after the initial diagnosis—from July to October 2020. A total of 148 patients were excluded from the study for the reasons set out in Fig. 1. The median and interquartile range for age and BMI were 59 (49–68) and 28 (26–32), respectively. Active smokers or ex-smokers 89 (41%) with International Coalition Against Tobacco (ICAT) of 0[1–2]. 52.6% had been in contact with family members with suspected or confirmed COVID-19. At the FFuC, the most prevalent symptoms were dyspnea in 138 (53.6%) together with fatigue116 (53.5%), emotional affectation 117 (53.9%) and depression 124 (57.1%). In 64 patients (30.3%), the abnormal radiological findings continued. These and those with stress dyspnea were asked for chest HRCT. In the SFuC, 154 patients (73.3%) still showed symptoms or claimed to develop new symptomatology after the acute process that was not attributable to alternative diagnoses. Dyspnea 88 (42.5%), fatigue 99 (47.8%), hair loss 47 (22.7%), emotional affectation 91 (44%), and depression 45 (21.7%) were the most frequent symptoms. However, other alterations such as memory, concentration, and language deficits started to appear after the FFuC, reflecting a global cognitive deficit of up to 56 (27.1%). They expressed it as a lack of mental fluency with stuttering and "brain fog"30. Also, erectile dysfunction or decreased sexual appetite was present in 3 (1.4%)—not plotted. The overall results of the FFuC and SFuC are shown in Figure S1 of the supplementary material.

Clinical outcomes and laboratory indices in female and males

Figure 2 shows the clinical features, physical examination, and mental health of females/males with PCS—red affected and blue healthy—for first/second follow-up consultations. At the FFuC, the most frequent symptom—over 25%—in females [males] were dyspnea in 69 (68.3%) [69 (40.5%)], fatigue 61 (60.4%) [55 (47.4%)], emotional affectation 60 (59.4%) [57 (49.1%)], and depression 36 (35.6%) [88 (75.9%)], see Fig. 2A. Note here that in

females, the symptoms of dyspnea, fatigue, and emotional affectation have a greater influence than in males, except for depression which affects more males than females. There was a gender difference for symptoms of arthralgia, fever, and hair loss. The rest of the less frequent symptoms—except epiphora—were still present after six months of the acute process. Figure 2B shows the results for the SFuC where the significant features for females [males] were dyspnea 46 (48.4%) [42 (37.5%)], fatigue 58 (61.1%) [41 (36.6%)], hair loss 42 (44.2%), emotional affectation 52 (54.7%) [39 (34.8%)], and depression 34 (35.8%). All these symptoms are more frequent in females than in males. From the p-values, we conclude that whether a person presents a lack of energy, emotional affectation, depression, or cognitive deficit depends on the person's gender. Also, decreased sexual appetite was observed in 1 female patient and erectile dysfunction in 2 males—not plotted.

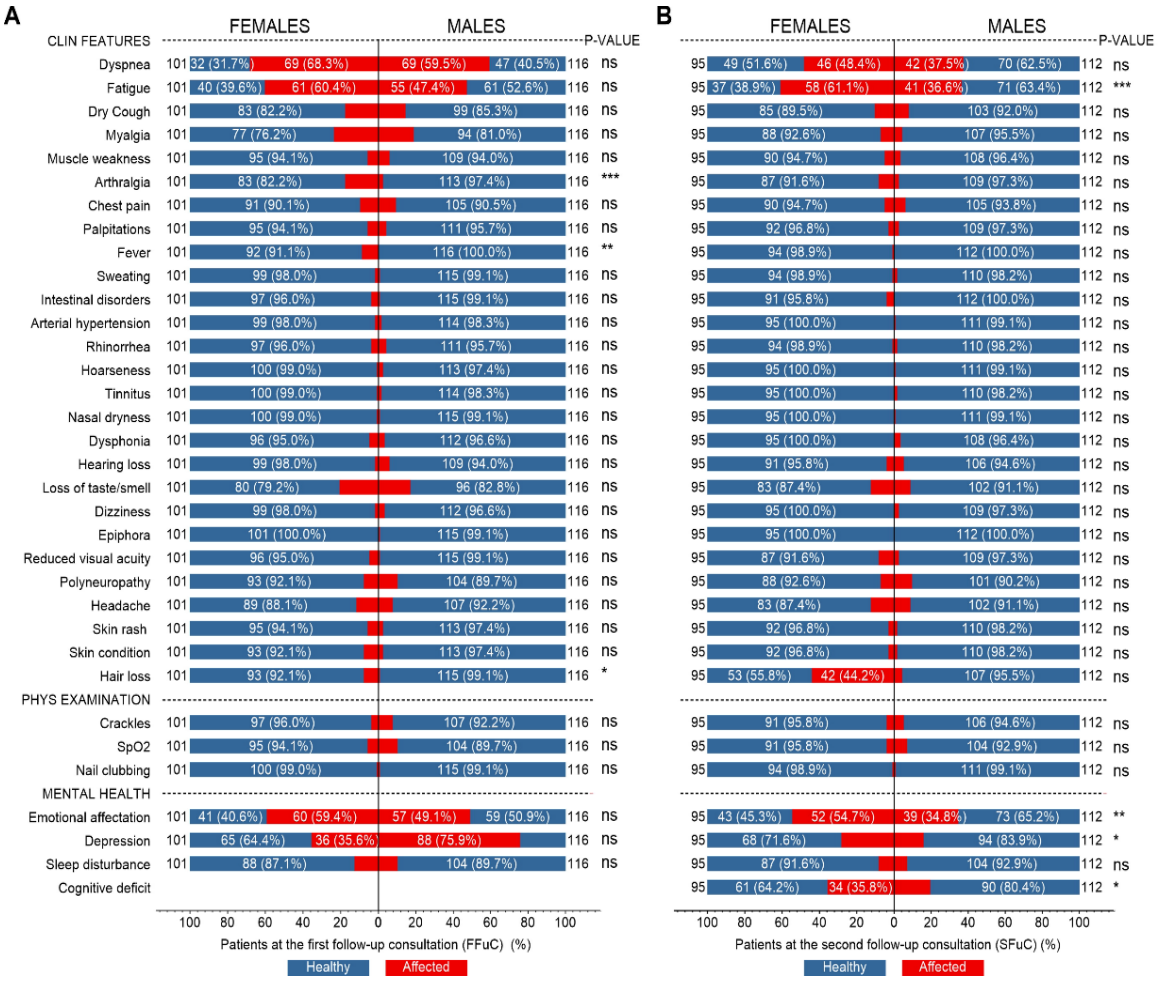

Figure 2: Clinical features, physical examination, and mental health of females and males with post-acute sequelae of SARS-CoV-2 infection for the first and second follow-up consultations — 2- and 6-months post symptom onset of COVID-19. Frequencies of symptoms presented in Nº (%) of the total for each gender in the FFuC **(A)** and SFuC **(B)**.

The normal (N, green color) and abnormal (Abn., yellow color) laboratory indices—antibodies, hematologic, biochemical, infection and coagulation—are shown in Fig. 3 for females and males. At the FFuC—Fig. 3A, a positive IgG and IgM were presented in 77 (80.2%) [95 (90.5%)] females [males] patients. This result confirms that both women and men have passed a relatively recent infection and are developing antibodies—as expected. A negative IgG and positive IgM or a positive IgG and negative IgM were presented in less than 25% of females and males. Also, Fig. 3A,C show this cohort's most relevant abnormal indices in females [male] patients. Hemoglobin 34 (31.8%) with 16 (15.7–16.4)—men only, creatine 26 (30.2%) with a median of 0.62 (0.57–0.64), ferritin 25 (32.1%) [73 (70.9%)] with 160 (132–213) [259 (173–405)] and D-dimer 26 (38.2%) [36 (38.3%)] with 0.97 (0.66–1.9) [0.83 (0.68–1.6)]. The hemoglobin and serum ferritin indices reject the chi-square null hypothesis of independence regarding gender. The antibody test of the SFuC—Fig. 3B—shows that a positive IgG and IgM were presented in 44 (48.9%) [47 (45.6%)] females [males] patients. Also, 40 (44.4%) [51 (49.5%)] of females [males] were positive with IgG and negative IgM, e.i., the patients have been in contact with the virus and have generated antibodies after six months. Here all the frequency of abnormal laboratory indices for females is less than 25%. However, more than one-quarter of male patients had abnormal hemoglobin [49 (47.6%) with 16.2 (15.7–16.6)] and more than half abnormal ferritin [57 (57%) with 198 (163–328)]. The hemoglobin, serum ferritin indices—similar to the FFuC, and the total bilirubin reject the chi-square null hypothesis of independence regarding gender. The median and the interquartile range for all the laboratory indices for females and males are represented in Fig. 3C. Here, the abnormal values of the indices for the different genera are clearly shown.

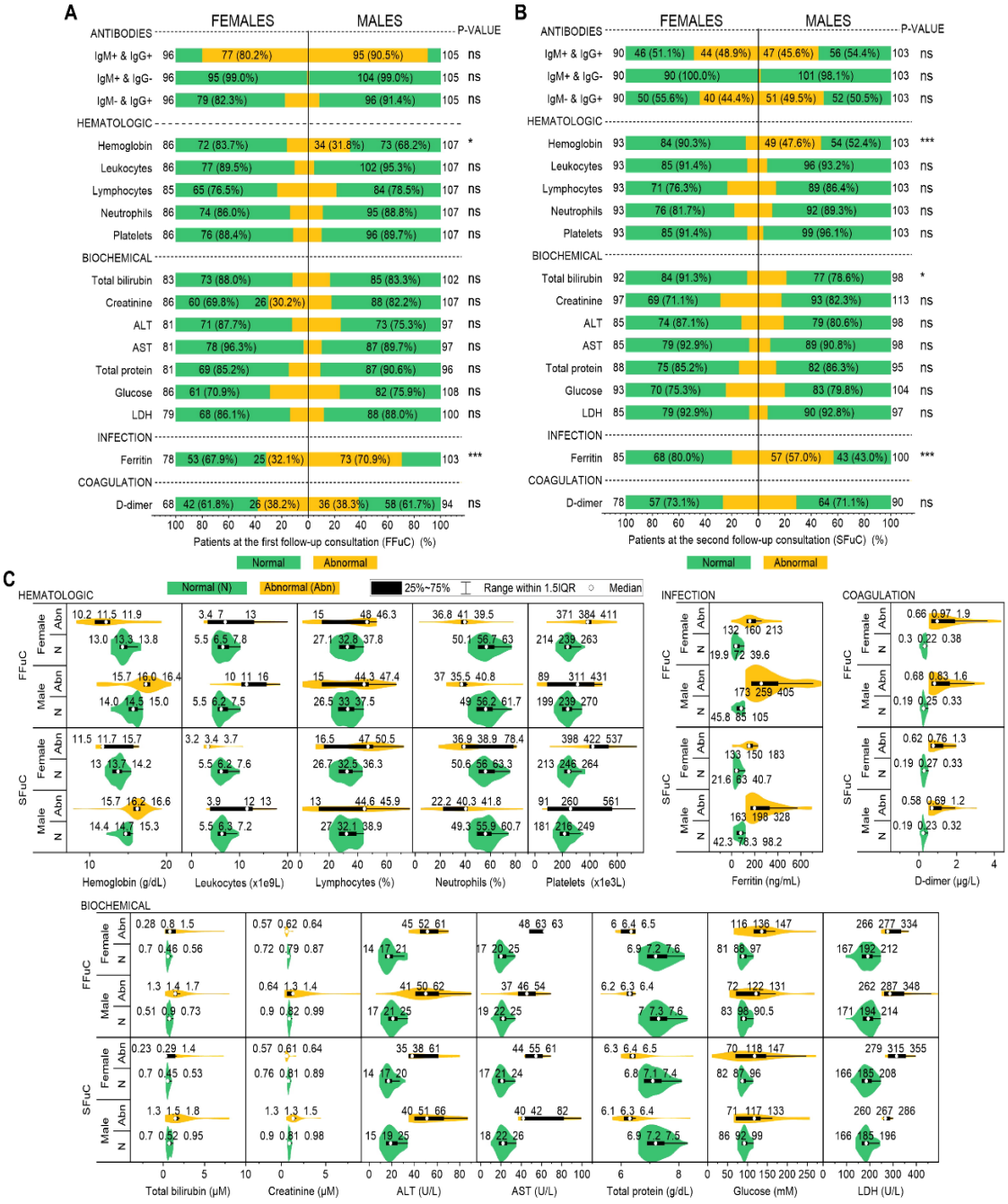

Figure 3: Laboratory indices of females and males with post-acute sequelae of SARS-CoV-2 infection for first/second follow-up consultations. Frequencies of normal (green color) and abnormal (yellow color) laboratory indices presented in Nº (%) of the total for each gender for the FFuC **(A)** and SFuC **(B)**. **(C)** Violin plots show the distribution, median, and quartiles of the laboratory indices for FFuC, SFuC, and gender.

Association between PCS features with PRD and hospitalization

Figure 4 shows the OR of PCS at 6-month follow-up using BVA and MVA, given the presence of a pre-existing respiratory disease (PRD) and hospitalization. A total of 207 patients were used for the BVA of gender, demographic characteristics, clinical features, mental health diseases, and hospitalization, of which 46 had the PRD. Also, we applied a BVA for lung exploration tests in a total of 157 patients, of which 33 had a PRD. For the MVA, we used 155 patients for all predictors, of which 33 had a pre-existing respiratory disease (see Fig. 4A). In addition, two hundred seven patients were used for the BVA with the same variables—except hospitalized—used for PRD analysis, of which 173 had been hospitalized. Also, for the BVA of the lung examination tests, 130 patients—of 157—had been admitted to the hospital. The MVA included 33 patients—of 155—that were discharged from the hospital (see Fig. 4B). The descriptive analysis, medians, and IQR of all the variables are shown in Figures S2 and S3 of the Supplementary Information.

After the bivariate adjustment, the following variables are in the robust (i.e., $2 \leq OR$) range of ORs positively associated with a PRD compared to those without PRD: abnormal BMI, dyspnea, fatigue, emotional affectation, depression, impairment of FVC, FEV1, VR, DLCO, and FEV1/FVC—below their normal limits. The remaining features are in the range of less impressive OR values (i.e., $0.5 < OR < 2$). Note that the highest OR corresponds to the FEV1/FVC ratio, implying that it has the highest bivalent association with the PRD compared to without PRD. Only the ORs of the abnormal BMI, dyspnea, fatigue, emotional affectation, depression, FEV < 80%, DLCO < 80%, and FEV1/FVC < 70% ratio are statistically significant (p values < 0.05). The ORs for abnormal BMI, ex-smokers, non-health workers, dyspnea, dry cough, loss of taste/smell, headache, emotional affectation, depression, all the impaired lung exploration tests, and the positive hospitalized status are in the positive or negative (i.e., $2 \leq OR$ or $OR \leq 0.5$) robust association range. The ORs of the remaining predictors are in a non-robust range. Statistically significant associations were found for abnormal BMI, ex-smokers, and dyspnea, as shown in Fig. 4A.

After the BVA, in the range of robust OR scores positive associated to hospitalized compared to non- hospitalized patients are the following features: male sex, age > 59 years, active-smoker, ex-smokers, non- health worker, the impairment of FVC, FEV1, and 6MWT. Venous thrombosis is the only predictor with a negatively associated with hospitalized patients. The explanation for this result is that pharmacological thromboprophylaxis was provided during hospitalization. The remaining variables after the BVA are in the less impressive range of ORs, implying a negative association with hospitalized patients compared to non-hospitalized

patients. Statistically significant associations were found for gender, age > 59 years, non non-health workers, and venous thrombosis (see Fig. 4B). After the MVA, the values of ORs for the male sex, non- health workers, age < 59 years, fatigue, loss of taste/smell, headache, hair loss, venous thrombosis, depression, impairment of FEV1, KCO, and 6MWT are in the range of strongly associated scores to the hospitalization status. The remaining variables are in the interval of non-robust OR values. Predictors of males, non-health workers, fatigue, and venous thrombosis contribute significantly to the MVA (see Fig. 4B).

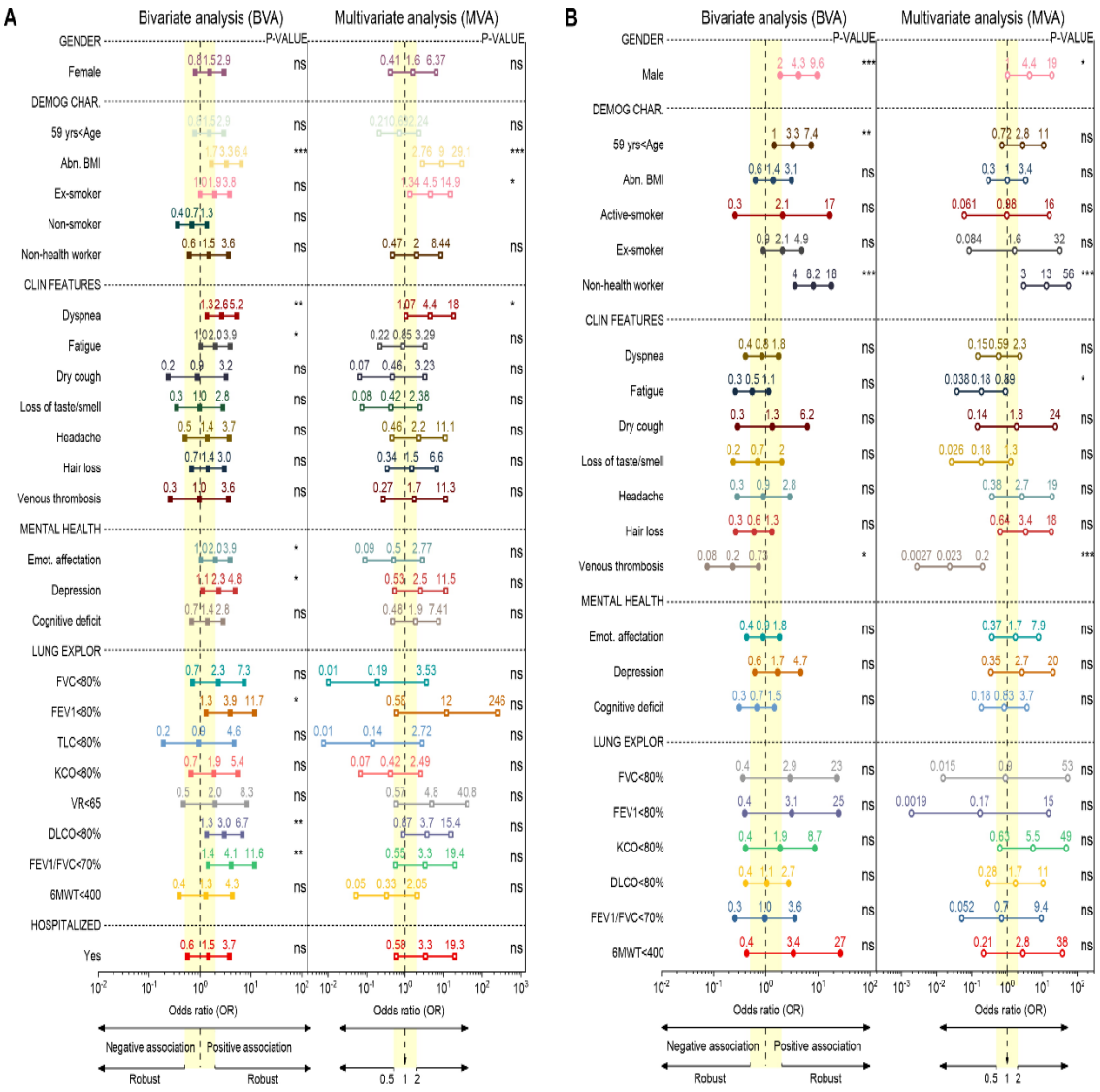

Figure 4: Forest plot of the odds ratio and its CIs (95%) values for the bivariate and multivariate analysis. Relationship between the PASC features with the pre-existing respiratory disease **(A)** and hospitalization **(B)**. The vertical yellow band delimits the regions for a robust (OR≤0.5 or 2≤OR) or weak association (0.5<OR<2). The p-values of the right column indicate the level of significance.

Association between PCS features with chest CT scan findings

The evolution of CT scans at the same level for a patient—a 65-year-old woman—at 0, 2, and 6 months after COVID-19 is depicted in **Fig. 5A–C**, respectively. At 0-months (Fig. 5A), the patient had patchy and bilateral ground-glass opacities (single arrows) and intralobular reticular pattern of peripheral subpleural distribution (double arrows). 2-months after COVID-19 (Fig. 5B), lesions have been significantly reduced but still have some residual reticular lesions (double arrows). Figure 5C shows the persistent subpleural banded reticular lesions (double arrow) in posterior segments of inferior lobes after 6-months of follow-up. See Figure S4A–D of the Supplementary Information for additional lung lesions from CT images of 4 patients.

Figure 5D shows the chest high-resolution CT findings of 130 patients, of which 74 (56.9%) presented chest image abnormalities due to consequences of COVID-19. The most notable—over 25%—features were: 68 (52.3%) had bilateral pulmonary involvement, 37 (28.5%) showed a subpleural reticular pattern, 56 (43.1%) had a peripheral distribution, and 59 (45.4%) exhibited a ground-glass opacity pattern. Less frequent CT findings were: unilateral lung involvement and interlobular reticular in 6 (4.6%) patients, central (8 [6.2%]) and central-peripheral (7 [5.4%]) lesion location, mixed GGO (3 [2.3%]), bronchiectasis (21 [16.2%]), emphysema (9 [6.9%]), PE (7 [5.4%]), laminar atelectasis (2 [1.5%]), and halo sign, pleural calcification, residual cavity, intrapulmonary node in 1 [0.8%] patient (see Fig. 5A).

Figure 5B shows the odds ratio after bivariate and multivariate logistic adjustments to measure the association of gender, demographic characteristics, lung examination, pre-existing diseases, and hospitalization status with the abnormal CT outcome. A total of 109 patients—of 130—were used for the BVA and MVA, of which 62 had abnormal CT results. The descriptive analysis, medians, and IQR of all the variables are shown in Figure S4 of the supplementary document. In bivariate analysis, males, age > 59 years, ex-smokers, abnormal radiography, impaired FEV1/FVC ratio, and a positive hospitalized status show a robust positive association (i.e., OR > 2). Non-smokers exhibit a robust negative OR, and the remaining variables are in the range of less impressive OR scores. Male sex, age > 59 years, non-smoker, ex-smoker, and abnormal radiography are statistically significant. Here the relationship of each characteristic is calculated separately with the CT results. After MVA, the ORs for the male sex, age > 59 years, former smoker, non-smoker, abnormal radiograph, DLCO < 80%, and FEV1/FVC < 70% in the robust interval of positively associated values. In addition, FVC < 80% and pre-existing respiratory disease are robustly adversely correlated with an abnormal CT outcome. The ORs for the remaining characteristics are in the weak

correlation range. Male sex, age > 59 years, and abnormal radiography were statistically significant.

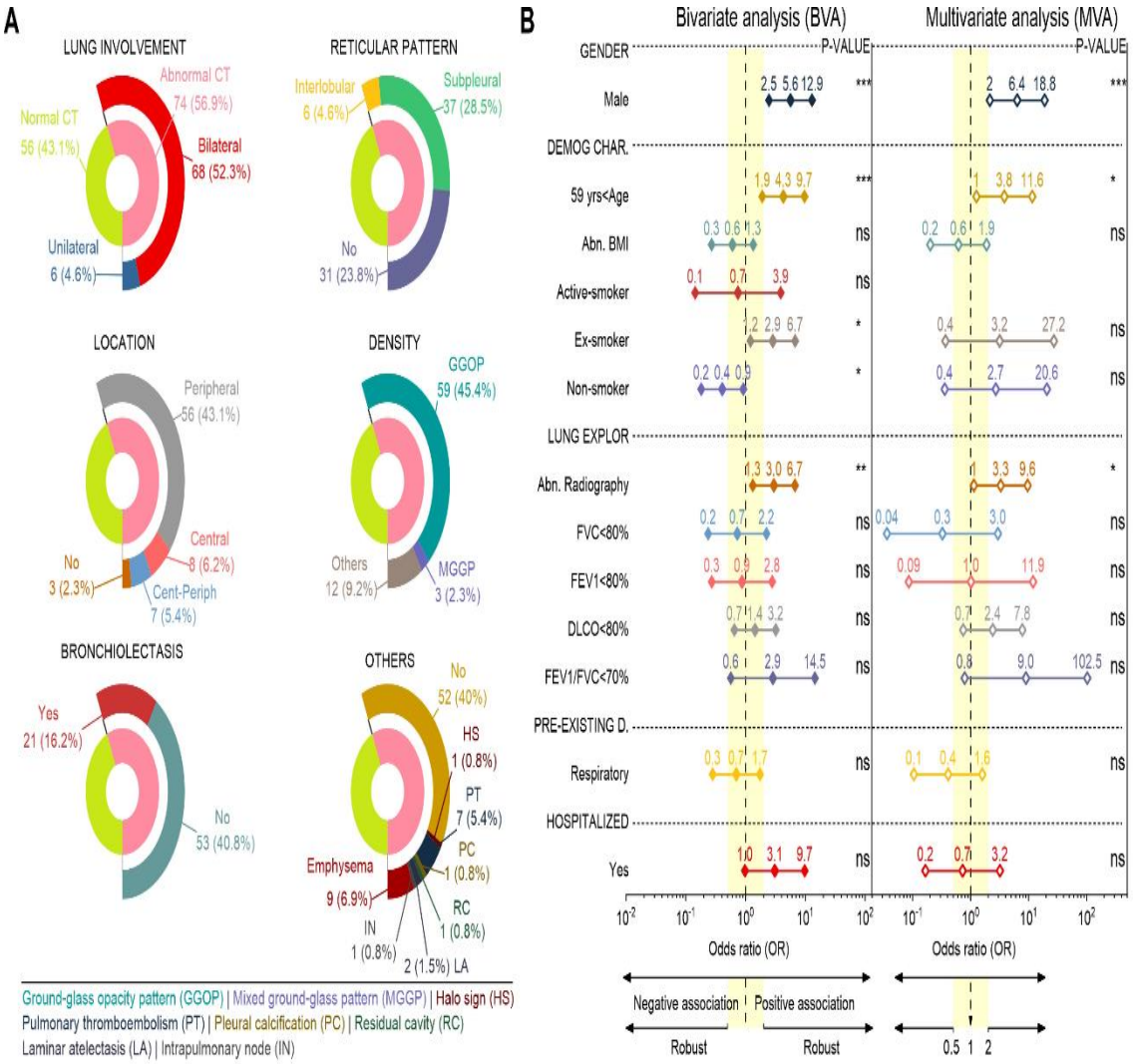

Figure 5: (A) Chest CT scan findings in all patients at the 6-month follow-up. **(B)** Forest plot of the odds ratio and its CIs (95%) values for the bivariate and multivariate analysis. Relationship between the PASC features with the normal/abnormal chest CT outcome. The vertical yellow band delimits the regions for a robust (OR≤0.5 or 2≤OR) or weak association (0.5<OR<2).

Discussion

We have presented quantitative analyses demonstrating the presence of PCS in patients at 6-month follow-up. We started with a descriptive analysis of the clinical, mental health, physical examination, and laboratory indices findings. We have shown that the most prevalent characteristics are: dyspnea, fatigue, dry cough, loss of taste/smell, headache, hair loss, emotional affectation, depression, and cognitive deficit for clinical and mental health

findings; and hemoglobin, lymphocytes, neutrophils, platelets, total bilirubin, creatine, ALT total protein, glucose, LDH, ferritin, and D-dimer for abnormal laboratory indices. This agrees with findings from previous long-term follow-up studies of SARS[11,13] and MERS[31,32]. In addition, we have shown that fatigue, arthralgia, fever, hair loss, emotional affectation, depression, cognitive deficit, hemoglobin, total bilirubin, and ferritin associated with PCS depend on the patient's gender. In particular, the female sex is favorable for the persistence of symptomatologies—matching prior findings[33].

At 6-months of follow-up, we found that the previous respiratory diseases and hospitalization status are strongly associated with specific demographic characteristics, clinical symptoms, mental health, and pulmonary function tests based on single and multiple PCS features. We found that PFTs were affected in patients with or without PRD—whether hospitalized or not-during the acute viral process and pulmonary involvement with restrictive and obstructive patterns and impaired diffusion capacity. The most frequent parameter to be highlighted was the diffusion capacity impairment. Its decrease may suggest an incipient DILD or the presence of pulmonary vascular abnormalities secondary to COVID-19. The bivariate analysis demonstrated the robust statistically significant association of patients with previous respiratory diseases with the following essential features: abnormal BMI, as a demographic characteristic, dyspnea and fatigue as clinical features, emotional affectation and depression as psychological complications, and impaired of FEV1, DLCO, and the FEV1/FVC ratio (i.e., positive diagnostic of obstructive and restrictive lung disease). However, after using more than one independent PCS feature, only the abnormal BMI, ex-smoker, and dyspnea had a robust statistically significant association to patients with PRD. This result implies that patients with PRD with one or more PCS features need to be monitored on a long-term follow-up basis. The following characteristics were obtained from the bivariate analysis for hospitalization status with a robust association and significant relationships: male sex, older than 59 years old, non-health worker, and venous thrombosis. Nevertheless, after the multivariate analysis, the robustness of the association for the predictors increased—except for the age > 59 years, adding fatigue as a new significant characteristic. The influential negative association of thromboembolic events—OR $\ll 1$—and fatigue suggests that patients without hospitalization also need long-term follow-up.

Our findings show the positive association between abnormal BMI and hospitalization patients for COVID-19—matching previous results[34], adding the new association with the presence of PRD. Regards to this, there is a debate on changing the relationship on BMI fluctuation of patients with overweight or obesity hospitalized for COVID-19 during their

follow-up. We claim that nutritional management strategies[35,36] during hospitalization and after discharge must be implemented to improve short- and long-term follow-up outcomes considering the comorbidities—as Di Filippo and colleagues proposed[34].

The major strength of our study is the long-term follow-up of patients with the examination of all patients reported at 2 and 6 months, including hospitalized and non-hospitalized patients. This study is the first to present single and multiple characterizations of the long-term sequelae of COVID-19. Moreover, our study is, to date, one of the most detailed and most prolonged follow-up studies of post-COVID-19 patients. However, the greater willingness of symptomatic patients to participate in a follow-up study is a possible biasing factor—as in all observational studies. The study findings may be limited due to the single-center, nonblinded, and nonrandomized design. We understand this potential localization bias.

Conclusions

At 6-months follow-up, PCS characteristics fatigue, arthralgia, fever, breathlessness, emotional disturbance, depression, cognitive deficit, hemoglobin, total bilirubin, and ferritin are correlated with the gender of the patient. Patients with previous respiratory diseases and abnormal body mass index, ex-smoker, and dyspnea had a robust statistically significant association. Non-hospitalized patients may suffer more severe thromboembolic events and fatigue than hospitalized patients. Functional lung tests are good predictors of chest CT imaging abnormalities in elderly patients with PCS.

The preliminary study presented here can be extended in several ways. First, the study can be prolonged to 12, 24, and 36 months of follow-up. This will enable us to study the long-term effects of PCS and define different degrees of severity. Second, adding new variables to the study will allow us to create models to predict the most frequent symptoms for medical treatments. In fact, we are working to improve our study in these directions.

Data availability

Currently, the third follow-up consultation (one year after the disease) is being collected, which means that data cannot be shared. Once the data analysis process for each follow-up process is completed, the data and the implemented code could be shared on an internet hosting, such as GitHub repository.

Abbreviations

SARS-CoV-2:Severe acute respiratory syndrome coronavirus 2

SARS-1:Severe acute respiratory syndrome

MERS:Middle East Respiratory Syndrome

PCS:Post-COVID-19 syndrome

ICAT:Coalition Against Tobacco

BMI:Body mass index

FFuC:First follow-up consultation

SFuC:Second follow-up consultation

X-Ray:Chest radiograph

CT:Chest computed tomography

HRCT:High-resolution computed tomography

GGO:Ground glass opacity

DILD:Diffuse interstitial lung disease

PFT:Pulmonary function test

FEV1:Forced expiratory volume in the first second

FVC:Forced vital capacity

FEV1/FVC:The FEV1/FVC ratio

RV:Residual volume

FRC:Residual functional capacity

TLC:Total lung capacity

DLCO:Carbon monoxide transfer by a single breath

KCO:Diffusion constant for carbon monoxide

6MWT:6-Minute walking test

SERAM:Spanish Society of Medical Radiology

IQR:Interquartile range

CIs:Confidence intervals

ORs:Odds ratios

BVA:Bivariate analysis

MVA:Multivariate analysis

PRD:Pre-existing respiratory disease

RT-PCR:Reverse transcription-polymerase chain reaction

PE:Pulmonary embolism

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Contributions

C.M.N. proposed and directed the research with help from B.M.J.R., J.G.F., and R.F.; M.I.S.P., and M.J.M.L. carried out the measurement and data collection of the pulmonary function tests; B.M.J.R. collected the demographic, clinical, and psychological data in follow-up consultations supported by P.I.G.F. and E.M.R.U.; G.L.M. and B.M.J.R. performed the acquisition and processing of HRCT images; J.G.F. undertook the processing of the

laboratory indices; R.F. and B.M.J.R. conducted the data analysis in Python; C.M.N., B.M.J.R., J.G.F., and R.F. wrote the manuscript; and all authors contributed to discussions**.**

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Supplementary Materials for

On the single and multiple associations of COVID-19 post-acute sequelae: 6-month prospective cohort study.

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S1. Clinical features, physical examination, psychology affectations, and laboratory indices.

Figure S1 shows a descriptive analysis of gender, physical examination, mental health, and laboratory indices for the first and second follow-up consultations. Note here that the violin plots clearly show the degree of severity in the laboratory results.

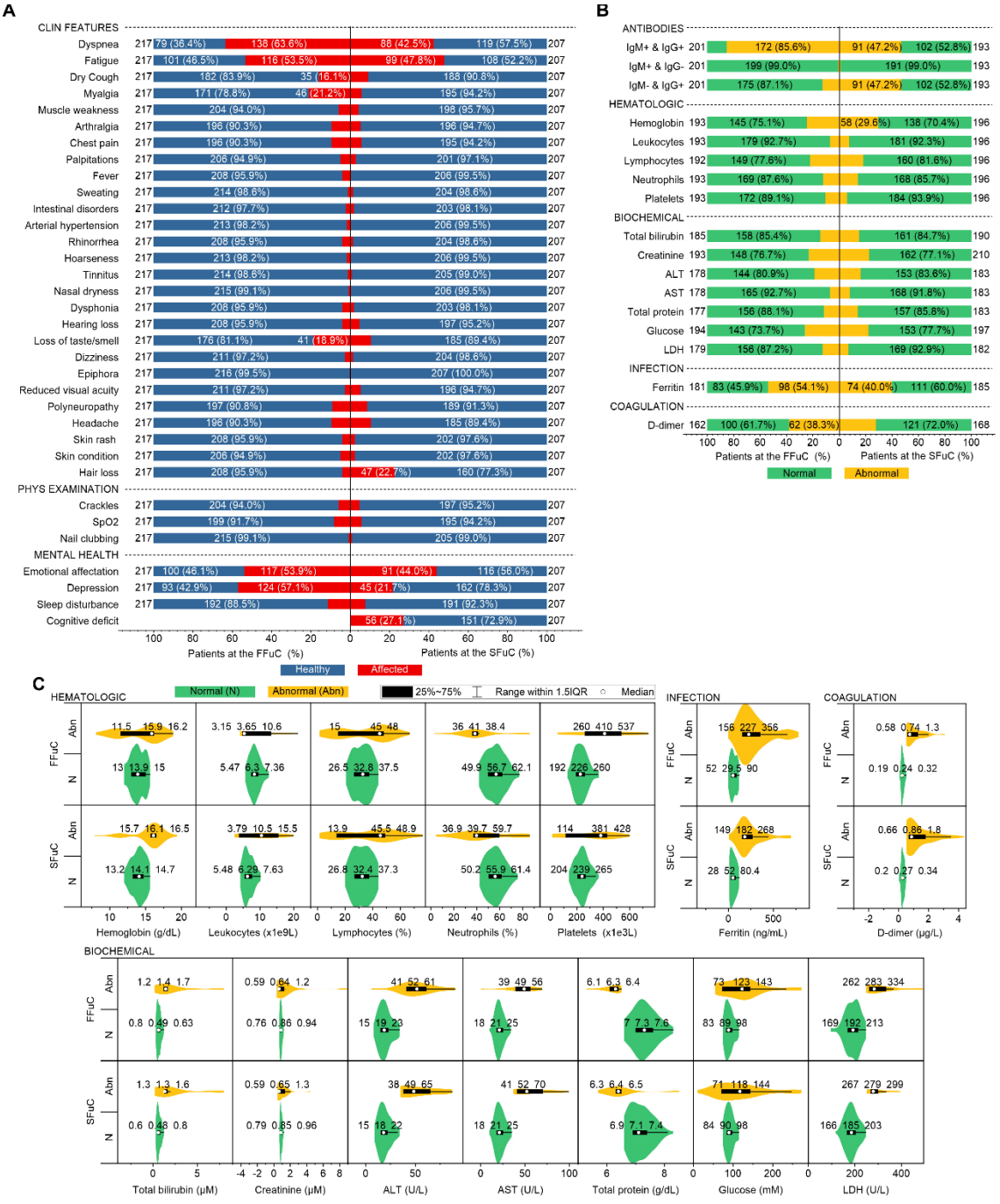

Figure S1. (A) Clinical features, physical examination, and mental health of patients with PCS for first/second follow-up consultations. **(B)** Frequency presented in Nº (%) for the laboratory indices of patients with PCS for first/second follow-up consultations. **(C)** Violin plos plots show the distribution, median, and quartiles of the laboratory indices for first/second follow-up consultations.

S2-S3. Pre-existing respiratory disease and hospitalization.

Figure S2 and **S3** show a descriptive analysis for gender, demographic characteristics, clinical outcomes, respiratory function tests, and mental health for patients with and without a PRD, as well as hospitalized and non-hospitalized.

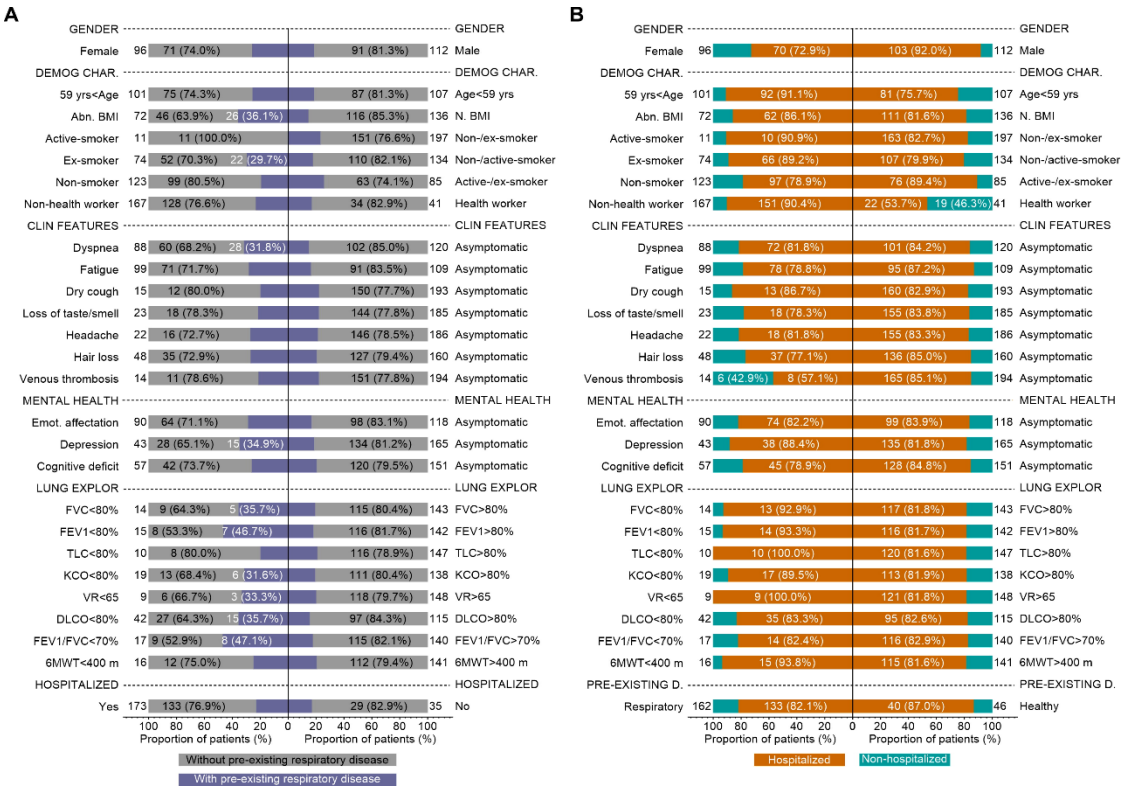

Figure S2. Frequency presented in Nº (%) of patients by gender, clinical and demographic characteristics, mental health impairment, and pulmonary function tests with/without a pre-existing respiratory disease **(A)** and hospitalized/non-hospitalized **(B).**

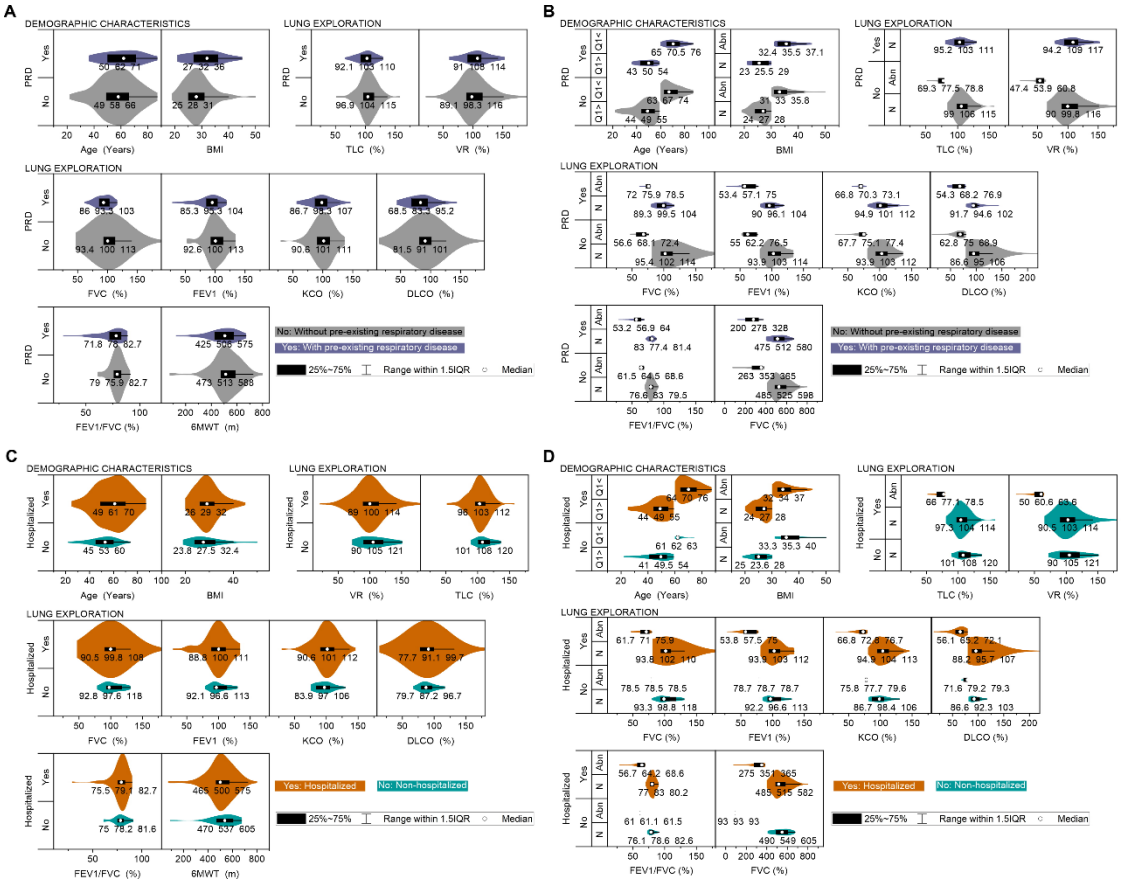

Figure S3. Violin plots show the distribution, median, and quartiles of the laboratory indices for first and second follow-up consultations of patients with/without a pre-existing respiratory disease **(A)**-**(B)** and hospitalized/non-hospitalized **(C)**-**(D)**.

S4. Chest high-resolution CT scan findings and laboratory reference ranges.

Figures S4A-B show the CT scans images of 4 patients after 6-months of follow-up. A 66-year-old man — BMI=32.8 and with PRD — with peripherally distributed GGOs (single arrow) and reticular pattern with architectural distortion of the parenchyma and focal traction bronchiectasis (double arrow) (Fig. S4A). A 58-year-old woman — BMI=28 and without PRD — with subpleural bands in both lungs, predominantly bibasal and primarily right (double arrow) (Fig. S4B). A 74-year-old man — BMI=30.1 and without PRD — with diffuse GGOs (single arrow) and reticular pattern with thickening of peripherally distributed interlobular septal (double arrow) (Fig. S4C). A 70-year-old man — BMI=30.4 and without PRD — with slight peripheral subpleural reticulation with small bronchiectasis (double arrow) (Fig. S4D). Figures S4E-F display a descriptive analysis of gender, demographic characteristics, lung function tests, PRD conditions, and hospitalized status.

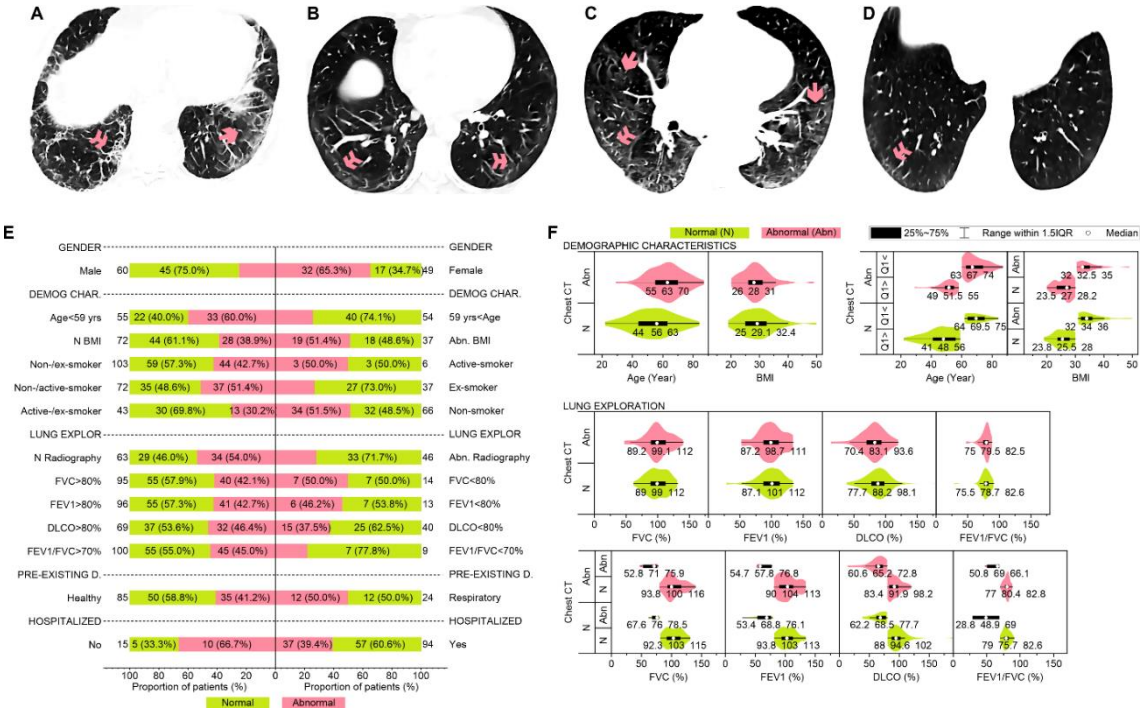

Figure S4. CT scan images of 4 patients **(A)-(C),** and findings in all patients at the 6-month follow-up. **(E)** Stacked bars showed the frequency in Nº (%) of patients for each feature with normal (green color) and abnormal (pink color) CT. **(F)** Violin plots show each demographic characteristic's distribution, medians, and IQR and lung exploration test.

7.2 Artículo 2. Evidencias de calidad.

[^{18}F]FDG PET/CT IN SHORT-TERM COMPLICATIONS OF COVID-19: METABOLIC MARKERS OF PERSISTENT INFLAMMATION AND IMPAIRED RESPIRATORY FUNCTION

(PET/CT con [18F]FDG en las complicaciones a corto plazo de COVID-19: Marcadores metabólicos de inflamación persistente y deterioro de la función respiratoria).

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[^{18}F] FDG PET/CT in Short-Term Complications of COVID-19: Metabolic Markers of Persistent Inflammation and Impaired Respiratory Function.

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Abstract

SARS-CoV-2 virus infects organs other than the lung, such as mediastinal lymph nodes, spleen, and liver, but, to date, metabolic imaging studies obtained in short-term follow-ups of patients hospitalized with severe COVID-19 infection are rare. Our objective was to evaluate the usefulness of [^{18}F] FDG-PET/CT in the short-term follow-up of patients admitted for COVID-19 pneumonia and to explore the association of the findings with clinical prognostic markers. The prospective study included 20 patients with COVID-19 pneumonia (November 2020-March 2021). Clinical and laboratory test findings were gathered at

admission, 48-72 h post-admission, and 2-3 months post-discharge, when [^{18}F] FDG-PET/CT and respiratory function tests were performed. Lung volumes, spirometry, lung diffusion capacity for carbon monoxide (DLCO), and respiratory muscle strength were measured. Volumetric [^{18}F] FDG-PET/CT results were correlated with laboratory and respiratory parameters. Eleven [^{18}F] FDG-PET/CT (55%) were positive, with hypermetabolic mediastinal lymphadenopathy in 90.9%. Mediastinal lesion's SUVpeak was correlated with white cells' count. Eleven (55%) patients had impaired respiratory function, including reduced DLCO (35%). SUVpeak was correlated with %predicted-DLCO. TLG was negatively correlated with %predicted-DLCO and TLC. In the short-term follow-up of patients hospitalized for COVID-19 pneumonia, [^{18}F] FDG-PET/CT findings revealed significant detectable inflammation in lungs and mediastinal lymph nodes that correlated with pulmonary function impairment in more than half of the patients.

Keywords: COVID-19; SARS-CoV-2; [18F] FDG-PET/CT; complications; inflammatory; respiratory function test.

1. Introduction

There is growing interest in the diagnosis, prognosis, and optimal clinical management of the sequelae of acute COVID-19 infection.

In the acute phase of infection, the epidemiology, clinical characteristics, results of standard clinical laboratory tests, lung CT appearance, treatment strategies, and outcomes in patients with COVID-19 have been reported in previous studies.Imaging techniques, especially high-resolution computed tomography (HRCT), have demonstrated a relevant diagnostic role [**2**], and multiple studies have been published on radiological findings in patients with COVID-19 pneumonia, especially during the acute phase and, more recently, over the short and medium terms [**2**,**3**].

The SARS-CoV-2 virus has been shown to infect organs other than the lung, such as the mediastinal lymph nodes, spleen, and liver, quantitative case studies in patients with COVID-19 are rare [**3**,**4**]. Such information can be obtained through the use of [^{18}F]- 2-Fluoro-2-Deoxy-Glucose ([^{18}F]FDG) positron emission tomography/computed tomography (PET/CT), which is commonly used to assess inflammatory and infectious lung diseases [**5**].

The complementary functional information provided by [^{18}F]FDG-PET/CT, which has been shown to be useful for diagnosing inflammatory and infectious lung diseases, estimating their severity, monitoring their evolution, and evaluating therapeutic response [**4**,**5**], can help elucidate the pathophysiological mechanisms of COVID-19. The value of [^{18}F]FDG-PET/CT has

been reported in patients with respiratory infections caused by other coronaviruses, such as MERS-CoV and SARS-CoV [**6**,**7**], as well as in patients with acute COVID-19 infection [**4**,**8**].

The [^{18}F]FDG-PET/CT studies of asymptomatic cancer patients described the incidental detection of interstitial pneumonia compatible with possible acute SARS-CoV-2 infection [**7**], and researchers have begun to examine the potential role of [^{18}F]FDG-PET/CT in its diagnosis and treatment [**8**]. As well as visual interpretation by an experienced specialist, [^{18}F] FDG-PET/CT also offers a semiquantitative approach to glycemic metabolism and, therefore, the intensity of inflammatory activity. Besides the standardized uptake value (SUV), recent studies in oncology have yielded additional parameters such as the metabolic tumor volume (MTV) and total lesion glycolysis (TLG) [**9**], which could be used to estimate inflammatory activity in lungs or extrapulmonary organs, especially lymph nodes. Studies of noncritical hospitalized patients have highlighted the possible relevance of lymph node hypermetabolism, quantified by the maximum SUV (SUVmax) in PET images, proposing that the highest SUVmax values for lesions and lymph nodes may indicate an increased severity of the infection and may predict a poor prognosis [**3**,**4**].

With this background, we hypothesized that [^{18}F]FDG-PET/CT could be useful to characterize pulmonary sequelae of COVID-19 infection. The objective of this study was to evaluate the usefulness of [^{18}F]FDG-PET/CT in the short-term follow-up of patients admitted for COVID-19 pneumonia and to explore the association of findings with clinical prognostic markers

2. Materials and Methods

2.1. Patients

This prospective, longitudinal, observational study enrolled consecutive COVID-19 patients at their follow-up visit 1–2 months after discharge from a third-level hospital between 27 November 2020 to 1 March 2021.

Study inclusion criteria were confirmation of COVID-19 in accordance with WHO guidelines [**10**] by a positive RT-PCR result for nasopharyngeal swabs, hospital admission between November 2020 to March 2021 (dates of “third wave” in Spain), and findings of ground-glass opacity or consolidation on chest HRCT scan or X-ray at admission. Exclusion criteria were age under 18 years, absence of microbiological confirmation of COVID-19 infection, history or presence of pulmonary fibrosis, active or uncontrolled COVID-19 infection at the time of the [^{18}F] FDG-PET/CT study, history or suspicion of oncological disease, pregnancy, and inability to sign informed consent.

The study was approved by the local Research Ethics Committee, and written, informed consent was obtained from all participants. Personal protective equipment was available for all staff, and COVID-19 infection prevention guidelines were always rigorously followed [**11**].

2.2. Clinical Information and Laboratory Test Results

For all patients, data were gathered from electronic medical records, including the results of clinical and laboratory tests at admission, at 48–72 h post-admission, and at the follow-up PET/CT examination. Analytical data included complete blood count, standard blood biochemistry, acute phase reactants, coagulation status [**12**], and neutrophil/lymphocyte ratios (NLRs). All patients underwent RT-PCR for nucleic acid testing of SARS-CoV-2.

2.3. Respiratory Function Tests

Respiratory function tests were performed at 2–3 months after hospital discharge. Spirometry results (in mL and % predicted) were obtained for forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and FEV1/FVC ratio. Body plethysmography was used to measure the residual volume (RV, in mL and % predicted), and total lung capacity (TLC, in mL and % predicted). The diffusing capacity of the lungs for carbon monoxide (DLCO) and the CO transfer coefficient (KCO) were expressed in absolute numbers and as % predicted. The results of the 6-min walk test (TM6M) were expressed as distance (in m) and % oxygen saturation at start and finish. Specifically trained personnel carried out functional tests using MasterScreen Body equipment (Jaeger, Hoechberg, Germany), considering reference values for the Mediterranean population and acceptability criteria established by European and Spanish regulations [**13**,**14**].

2.4. PET/CT Data Acquisition

After two consecutive negative RT-PCR test results for SARS-CoV-2 nucleic acid, confirming that patients were no longer infected, patients underwent [^{18}F] FDG-PET/CT imaging (Siemens Biograph Vision 600 PET/CT, Siemens Healthcare, Erlangen, Germany), always performed within 2–3 months after discharge from hospital. The test protocol was based on international recommendations [**15**]. Patients were administered intravenously with the radiopharmaceutical (3.7–4.81 MBq/kg) at rest after fasting for at least 6 h with adequate hydration as long as their capillary blood glucose level was below 6.8 mmol/L. Image acquisition (whole body in 3D) started at 50–60 min post-injection with the acquisition of a topogram (50 mA, 120 kV), followed by helical CT without contrast (170 mA, 120 kV) and the acquisition of PET images with coverage from skull base to mid-thigh.

2.5. PET/CT Image Interpretation

The [^{18}F] FDG-PET/CT and chest CT images were independently analyzed by two nuclear medicine physicians (E.M.T.I. and M.G.M.) with a great deal of experience in the interpretation of cardiothoracic images, using syngo.via version VB40B software (Siemens Healthcare, Erlangen, Germany). They were blinded to the biological and clinical data of patients. Discrepancies in interpretations were resolved by consensus with a third expert nuclear medicine physician (A.R.F.).

The [^{18}F] PET/CT data were transferred to a computer workstation (syngo.via) for the co-registration of PET and CT images. Regions of interest (ROIs) were drawn on CT images of lungs around areas with evident loss of aeration and adjacent areas of normal appearance. ROIs were also drawn on CT images of mediastinal lymph nodes. The ROIs drawn on the CT images of each patient were transferred to the co-registered PET images and the amount of [^{18}F]FDG pathological uptake was calculated for each ROI, determining maximum, peak, and minimum SUVs, normalized by body weight (SUVmax, SUVpeak, and SUVmin, respectively) and lean body mass (SUL); metabolic tumoral volume (MTV; volume of pixels in the ROI with SUVmax >40%); and total lesion glycolysis (TLG; MTV multiplied by SUVmean).

2.6. Chest CT and X-ray Image Interpretation

Upon their diagnosis, all patients underwent chest X-ray in posterior-anterior and lateral projections, reported by specialist radiologists according to current recommendations [**16**,**17**]. They characterized the density (alveolar, ground glass, or mixed), distribution (central, peripheral, or diffuse), location (unilateral or bilateral), and extent (unilobar or multilobar).

2.7. Statistical Analysis

All measurements for each participant were independently conducted by two nuclear medicine physicians, considering the mean value in statistical analyses. Absolute numbers and percentages were calculated for categorical variables and means with standard deviation (SD) for continuous variables. For comparisons of quantitative data between the positive and negative PET groups, the Student's *t*-test was applied when the distribution was normal and the Mann–Whitney U test when it was not. Associations with categorical variables were evaluated by constructing contingency tables, applying the chi-square test for individual comparisons and Fisher's exact test for multiple comparisons. Volumetric [^{18}F]FDG-PET/CT results were correlated with laboratory test results and respiratory function parameters by using Spearman's rank correlation coefficient. IBM SPSS version 15.0 (IBM Corp, Armonk, NY,

USA) and R software were used for statistical analyses. A $p \leq 0.05$ was considered significant in all tests.

3. Results

The study included 20 patients (60% males) with a mean age of 55.85 ± 9.28 years admitted for pneumonia and/or respiratory failure between 27 November 2020 and 1 March 2021 (during the "third wave" of COVID-19 in Spain). The mean hospital stay was 16.70 ± 11.99 days. **Table 1** summarizes the baseline characteristics of the patients.

Table 1. Baseline clinical characteristics and risk factors of patients.

Clinical Characteristics (n)	mean ± SD or n (%)
Age (years)	55.85 ± 9.28
Gender (Male)	12 (60)
BMI (kg/m^2)	34.11 ± 7.23
Comorbidities	
Former or current smoking habit	2 (10)
Hypertension	5(25)
Diabetes	3 (15)
Hyperlipidemia	2 (10)
Atrial fibrillation	2 (10)
Asthma	3 (15)
Charlson Comorbidity index	1.60 ± 1.14
Charlson Comorbility index ≥ 2	9 (45)
Clinical characteristics at admission	
Fever	17 (85)
Dyspnea	15 (75)
Irritative cough	16 (80)
Fatigue	14 (70)
Myalgia	11 (55)
Anosmia/ Ageusia	2 (10)

Digestive symptoms	9 (45)
Headache	3 (15)
ARDS (PaO_2/FIO_2 < 300 mmHg	14 (70)
Blood oxygen saturation	90.90 ±5.33
Laboratory test results at admission	
Hemoglobin (g/dL)	14.86 ± 1.84
White blood cell (count $x10^3/\mu L$)	7.68 ± 3.11
Neutrophil (count $x10^3/\mu L$)	6.28 ± 3.16
Lymphocyte (count $x10^3/\mu L$)	0.99 ± 0.57
NLR	8.36 ± 5.86
Platelet (count $x10^3/\mu L$)	206.65 ± 54.49
Ferritin (ng/mL)	1327.81 ± 1402.58
C-reactive protein (mg/L)	81.20 ± 54.61
LDH (U/L)	398.15 ± 113.80
AST(U/L)	49.25 ± 38.60
ALT(U/L)	48.30 ± 46.36
Albumin (g/dL)	3.92 ± 0.50
D-dimer (mg/L)	0.73 ± 0.52
Characteristics of Hospitalization	
Hospital stay (days)	16.70 ± 11.99
Pneumonia (chest X-ray)	19 (95)
ICU admission	10 (50)
Invasive mechanical ventilation	5 (25)
Bolus therapy with glucocorticoid	14 (60)
Antiviral therapy	5 (25)
Selective inhibitors of pro-inflammatory cytokines	6 (30)

Table 1. Continuous variables are presented as means ± standard deviation (SD) and categorical variables as frequencies (percentages). ARDS: acute respiratory distress syndrome, AST: aspartate aminotransferase, ALT: alanine transaminase, NLR: neutrophil/ lymphocyte ratio, PCT: procalcitonin, NT-proBNP: N terminal pro-B-type natriuretic peptide, LDH: lactate dehydrogenase, ICU: intensive care unit.

The main symptom at admission was fever in 17/20 patients (85%), followed by irritative cough in 16 (80%), dyspnea in 15 (75%), fatigue in 14 (70%), and ageusia and/or anosmia in 2 patients (10%). Chest X-ray findings compatible with pneumonia were observed in 19 patients (95%), being multilobar in 18 (97.4%) and unilobar in 1 (5.3%). The radiological pattern was alveolar in three patients (15.8%), ground glass in seven (36.8%), and mixed in the remaining nine (47.4%). The main associated complication during hospitalization was respiratory distress in 14 (70%) patients; admission to the intensive care unit (ICU) was required for seven (35%) of these patients and invasive mechanical ventilation in five (25%). All patients were treated with corticosteroids, administered as a bolus in 14 patients (70%). Five patients (25%) received antiviral treatment and another six (30%) were treated with selective inhibitors of pro-inflammatory cytokines (five with tocilizumab and one with anakinra). Finally, three patients (15%) required home oxygen therapy at discharge.

3.1. The [^{18}F]FDG-PET/CT Findings

The mean time from hospital discharge to [^{18}F]FDG-PET/CT study was 58.85 ± 13.67 days. The result was positive in 11 patients (55%) and negative in 9 (45%). The main finding was hypermetabolic lymphadenopathy in the mediastinum, observed in 10 (90.9%) of the [^{18}F]FDG-PET/CT-positive patients (**Figure 1**)

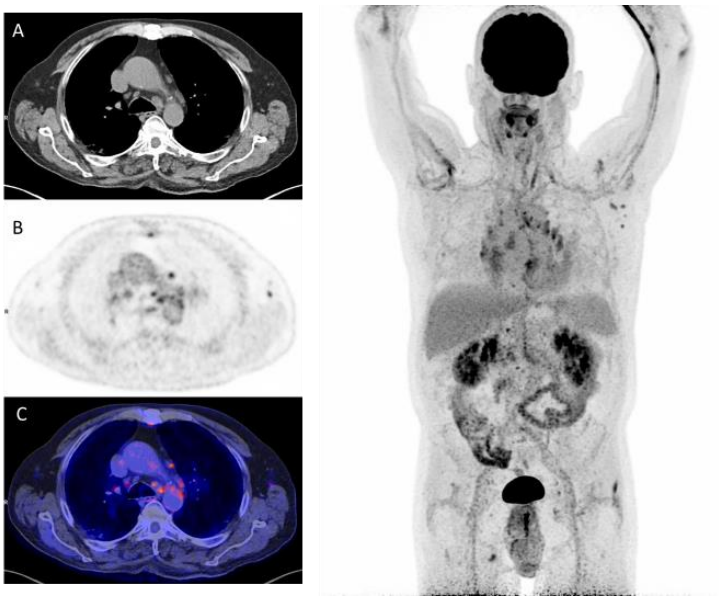
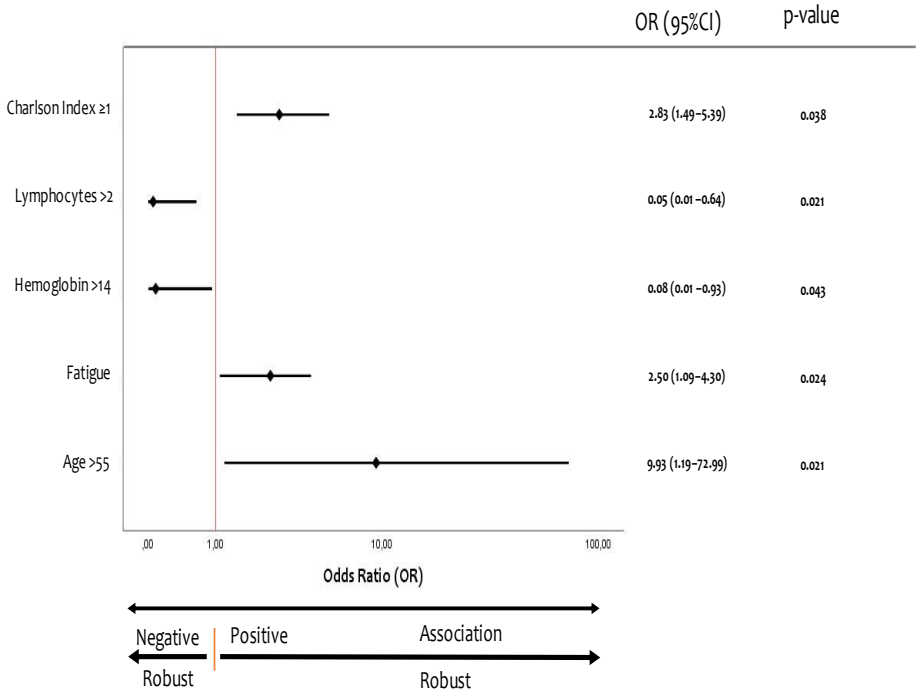


Fig. 1 A 64-year man patient admitted for multilobar pneumonia caused by SARS-Cov-2. ^{18}F-FDG PET/CT at 3 months after onset of symptoms shows increase FDG uptake in the residual pulmonary lesions (TLG 124,11) and in the mediastinum lymph node (SUVpeak 1,73). Pulmonary function tests showed severe pulmonary diffusion impairment with a diffusing capacity of the lungs for carbon monoxide (DLCO) of 41% predicted. Left: A) CT transverse slice, B) ^{18}F-FDG PET slice, and C) ^{18}F-FDG PET and CT-fused images. Right whole-body maximal intensity projection (MIP) image, displayingmediastinal lymph nodes ^{18}F-FDG uptake

Patients with positive and negative [^{18}F]FDG-PET/CT results significantly differed in age (59.82 ± 8.52 vs. 51.00 ± 8.09 years, respectively, p = 0.03), Charlson index score ≥1 (66.7 vs. 100%, p = 0.038), presence of fatigue (90.9 vs. 44.4%, p = 0.024) and respiratory distress (90.9 vs. 44.4%, p = 0.024), hemoglobin levels (13.41 ± 1.91 vs. 15.24 ± 1.58 g/dL, p = 0.041), and lymphocyte count (1.78 ± 0.53 vs. 2.47 ± 0.49 × 10^3/µL, p = 0.011) at 2–3 months post-discharge (**Figure 2**).

Fig. 2. Factors associated with FDG-PET/CT positive. Forest plot with odds ratios shown by closed circles and 95% confidence intervals by whiskers



Lymphocytes >2: Lymphocyte count < 2x10^3/µL; Hemoglobin >14: hemoglobin >14 ng/mL.

3.2. Correlation of Volumetric [^{18}F]FDG-PET/CT Parameters with Laboratory Test Results.

Table 2. Bivariate correlations of volumetric ^{18}F-FDG PET/CT parameters with laboratory parameters at admission, during hospital stay, and at 2-3 months post-discharge (short-term follow-up).

Variable	SUVpeak		Pulmonary TLG	
	Spearman´s Rho	p-value	Spearman´s Rho	p-value

Admission	Hemoglobin (g/dL)	-0.664	0.026		
	Neutrophil count	-0.764	0.006		
	Lymphocyte count	0.636	0.035		
	NLR	-0.664	0.026		
Hospital stay	Neutrophil count	-0.700	0.016		
	Lymphocyte count	0.618	0.043		
	NLR	-0.627	0.039		
	IL-6			0.624	0.010
	C-reactive protein			0.618	0.004
	PCT			0.570	0.049
	LDH			0.445	0.049
	Troponin			0.883	0.002
	Fibrinogen			0.635	0.015
	D-dimer			0.674	0.001
Short-term follow-up	Neutrophil count	-0.679	0.022		
	Lymphocyte count	0.791	0.004		
	NLR	-0.727	0.011		

Table 2: IL-6: interleukin 6; LDH: lactate dehydrogenase; NLR: neutrophil/ lymphocyte ratio; PCT: procalcitonin.

At admission, a significant correlation was found between the SUVpeak of the target lesion in the mediastinum and the hemoglobin level (r = 0.615, *p* = 0.044), leukocyte count (rho = −0.664, *p* = 0.026), neutrophil count (rho = −0.764, *p* = 0.006), lymphocyte count (rho = 0.636, *p* = 0.035), and NLR (rho = −0.664, *p* = 0.026). In addition, the TLG in lung parenchyma was significantly correlated with C-reactive protein (CRP) (rho = 0.558, *p* = 0.011),

procalcitonin (rho = 0.611, *p* = 0.035), fibrinogen (rho = 0.472, *p* = 0.041), and blood glucose (rho = 0.517, *p* = 0.020) levels at hospital admission.

During hospitalization, the SUVpeak of the target lesion was again significantly correlated with neutrophil count (rho = −0.700, *p* = 0.016), lymphocyte count (rho = 0.618, *p* = 0.043), and NLR (rho = −0.627, *p* = 0.039). Furthermore, pulmonary TLG was significantly correlated with IL-6 (rho = 0.624, *p* = 0.010), CRP (rho = 0.618, *p* = 0.004), procalcitonin (rho = 0.570, *p* = 0.042), LDH (rho = 0.445, *p* = 0.049), troponin (rho = 0.883, *p* = 0.002), fibrinogen (rho = 0.635, *p* = 0.015), and D-dimer (rho = 0.674, *p* = 0.001) levels and with the neutrophil count (rho = 0.615, *p* = 0.044), lymphocyte count (rho = −0.615, *p* = 0.004), and NLR (rho = 0.558, *p* = 0.011) during the hospital stay.

At the follow-up at 2–3 months, the SUVpeak was significantly correlated with neutrophil count (rho = −0.679, *p* = 0.022), lymphocyte count (rho = 0.791, *p* = 0.004), and NLR (rho = −0.727, *p* = 0.011). No significant correlation was found between pulmonary TLG and any analytical parameter under study (**Figure 3**).

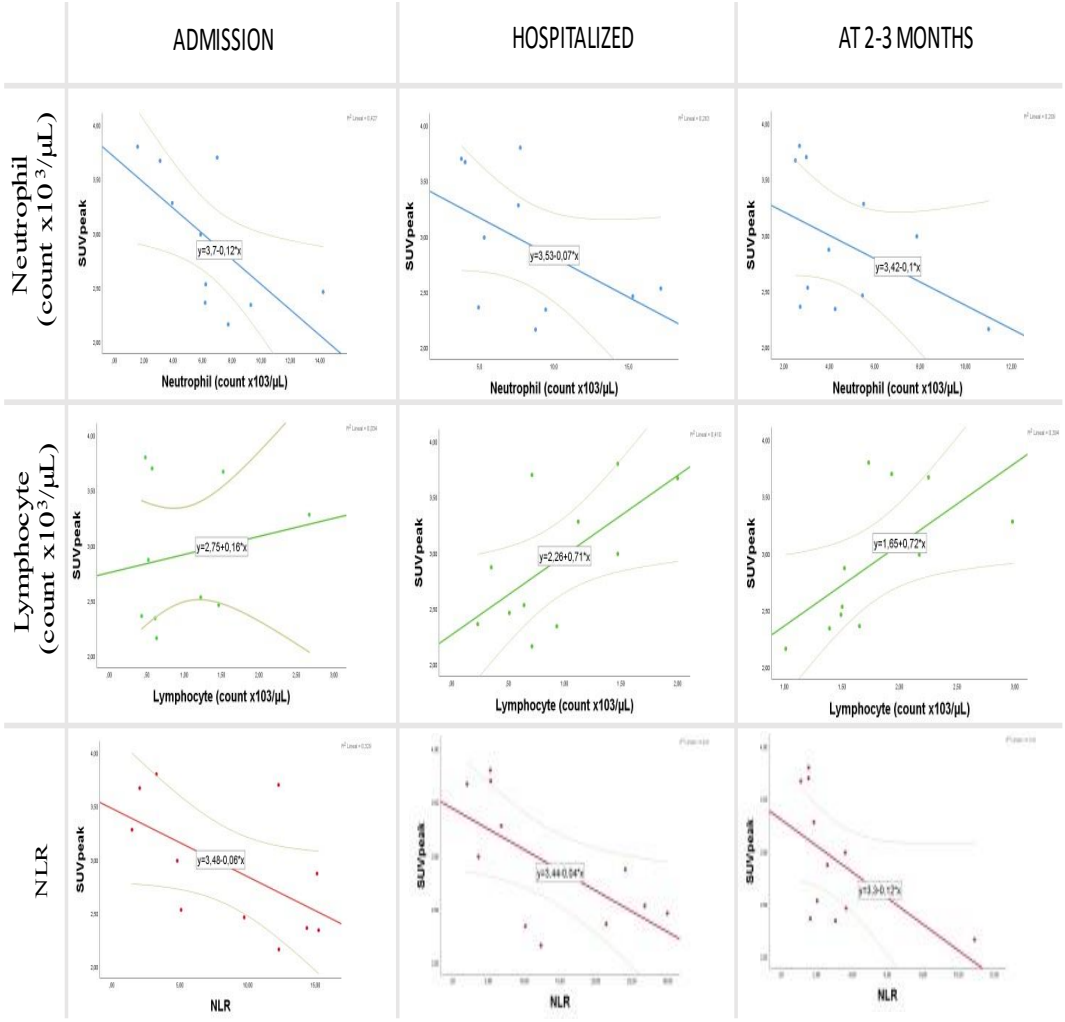


Figure 3. Scatter plot matrix. Correlation of the SUVpeak of the target lesion with neutrophil and lymphocyte counts and NLR at admission, during hospital stay, and at 2–3 months post-discharge. Figure 3. Scatter plot matrix. Correlation of the SUVpeak of the target lesion with neutrophil and lymphocyte counts and NLR at admission, during hospital stay, and at 2–3 months post-discharge.

3.3. Correlation of Volumetric [^{18}F]FDG-PET/CT Parameters with Respiratory Function Parameters

Eleven (55%) of the 20 patients had impaired respiratory function. Percentage predicted values were <80% for FVC in 20% of patients, <80% for FEV1 in 15%, <70% for FEV1/FVC in 5%, <80% for TLC in 20%, <80 for DLCO in 35%, <80% for KCO in 25%, and <65% for VR in 5%. Saturation was ≥4% lower at the finish versus start of the walk test in four patients (20%), and the distance was <400 m in three (15%).

Volumetric [^{18}F]FDG-PET/CT parameters were related to respiratory function test results obtained at 2–3 months post-discharge (**Figure 4**). The SUVpeak of the target lesion in the mediastinum was significantly and positively correlated with % predicted DLCO (rho = 0.782, p = 0.008), KCO (rho = 0.721, p = 0.019), and RV (rho = 0.636, p = 0.048) values. Pulmonary TLG was significantly and negatively correlated with % predicted DLCO (rho = −0.628, p = 0.005), KCO (rho = −0.564, p = 0.014), TLC (rho = −0.532, p = 0.023), and RV (rho = −0.554, p = 0.017) values. (**Table 3**).

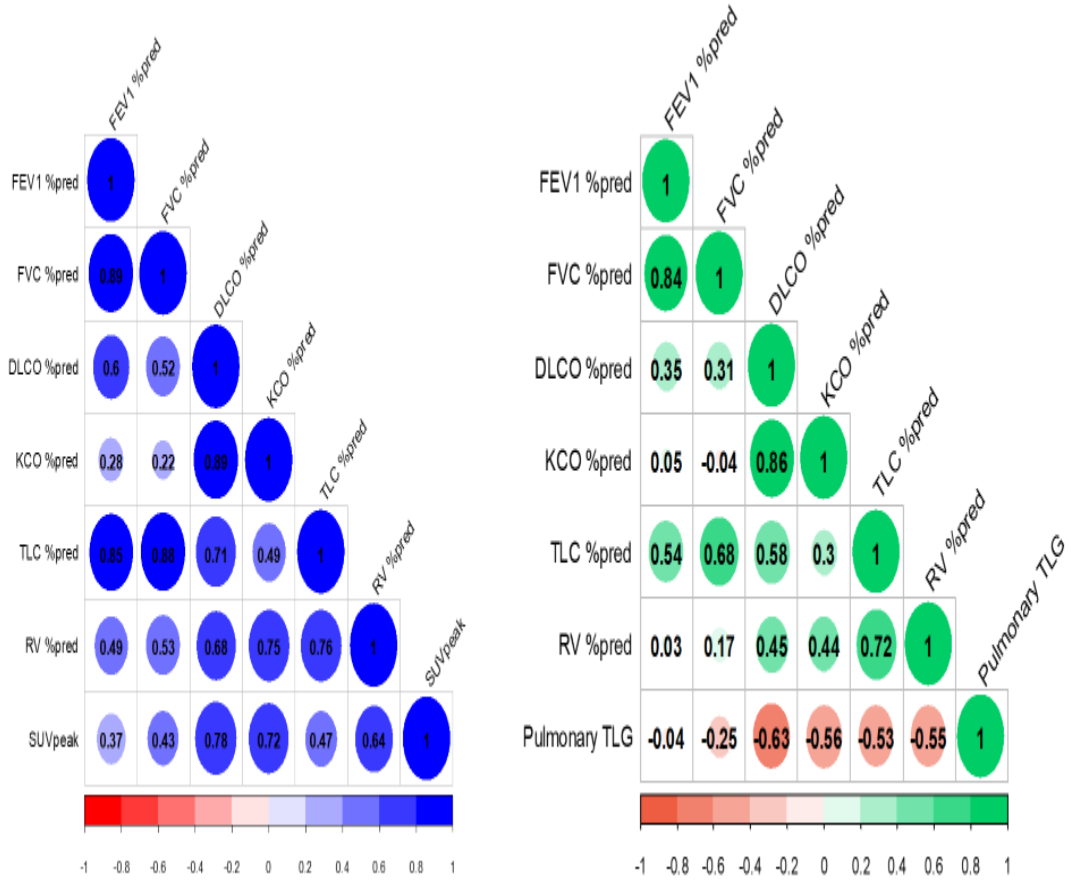
Table 3. Bivariate correlations of volumetric ^{18}F-FDG PET/CT parameters and respiratory function parameters in the short-term follow-up.

Variable	SUVpeak		Pulmonary TLG	
	Spearman's Rho	p-value	Spearman's Rho	p-value
DLCO % pred	0.782	0.008	-0.628	0.005
KCO % pred	0.721	0.019	-0.564	0.014
TLC % pred	0.467	0.174	-0.532	0.023

RV % pred	0.636	0.048	-0.554	0.017

Table 3: DLCO: diffusing capacity of the lungs for carbon monoxide; KCO: CO transfer coefficient; RV: residual volume, TLC: total lung capacity.

Fig. 4 Correlogram showing the association of respiratory function test results with the SUVpeak of the target lesion and pulmonary TLG



Color intensity and the size of the circle are proportional to the correlation coefficients.

4. Discussion

In this study, [^{18}F] FDG-PET/CT was used to measure the metabolism of lungs and other organs in the short–medium follow-up of patients admitted to hospital for pneumonia or respiratory failure due to COVID-19 infection. Despite testing negative for the infection in two successive RT-PCR tests of nasopharyngeal swabs, more than half of the patients showed increased metabolic activity (i.e., persistent inflammation) on [^{18}F]FDG-PET/CT images in lung tissue of normal appearance and in mediastinal lymph nodes. To our best knowledge, [^{18}F]FDG-PET/CT has not previously been used to detect residual inflammatory processes

after COVID-19 infection. These findings contribute evidence on the pathophysiological processes in patients who survive hospital admission for COVID-19 pneumonia.

The [^{18}F]FDG-PET/CT has been employed in patients with influenza A, aspiration pneumonia, and organized pneumonia to assess the extent and severity of the disease, to follow its course, and to evaluate the response to therapy [**5**,**18**,**19**]. Research on the role of [^{18}F]FDG-PET/CT in COVID-19 infection has generally focused on the acute phase. In this regard, Qin et al. reported high [^{18}F]FDG uptake in lung lesions and mediastinal lymph nodes of four patients strongly suspected of the infection [**4**], and Colandrea et al. described elevated [^{18}F]FDG uptake in lung lesions in 80% of a series of symptom-free oncology patients diagnosed with COVID-19 [**20**]. However, few studies have addressed the short- or medium-term consequences of COVID-19 infection. Dietz et al. recently reported increased [^{18}F]FDG uptake in lung lesions and mediastinal lymph nodes of 13 non-critically ill COVID-19 patients at days 6–14 after symptom onset, although the short-axis diameter of mediastinal lymph nodes was always < 1 cm [**3**]. Johnson et al. proposed that high [^{18}F]FDG uptake in mediastinal lymph nodes might be secondary to lung involvement in COVID-19 [**21**]. Bai et al. found elevated metabolic activity in residual lung lesions in COVID-19 survivors after two successive negative results in the RT-PCR test [**22**], and Scarlattei et al. reported that this metabolic activity remained high many weeks after the disappearance of symptoms and a negative RT-PCR test result [**23**]. The present results are in line with the above findings and contribute novel data on increased metabolic activity in lung tissue of normal appearance and in mediastinal lymph nodes of normal size. In this context, Xu et al. described lymphocyte-dominated interstitial mononuclear inflammatory infiltrates in both lungs of a patient with COVID-19 and reported that substantial inflammation may persist in the lungs after the disappearance of the infection [**24**]. The elevated [^{18}F]FDG uptake would reflect increased glycolytic activity due to infiltration and inflammation of the lung, even in normally aerated areas that show no morphological alterations on CT images, demonstrating the greater capacity of [^{18}F]FDG-PET/CT to detect inflamed lung areas in comparison to CT alone [**8**,**22**], which may persist long after the disappearance of COVID-19 infection. The possible duration of the post-COVID-19 inflammatory response in lungs and extrapulmonary sites has yet to be established and warrants further research.

At 2–3 months post-discharge, patients with elevated chest [^{18}F]FDG uptake were older and characterized by a higher Charlson index, more frequent fatigue and respiratory distress, and lower hemoglobin and lymphocyte counts in comparison to those with normal [^{18}F]FDG uptake. The SUVpeak of the target lesion and pulmonary TLG were significantly correlated

with acute phase reactants and white blood cell counts at admission, during the hospital stay, and at 2–3 months post-discharge. Although there is a lack of similar studies in severely ill COVID-19 survivors for comparison with these results, they are consistent with previous findings on risk factors for more severe infection, including old age, underlying comorbidities [**12**,**25**], and similar changes in white blood cell counts, lymphocyte counts, procalcitonin and CRP levels, and NLR [**26**,**27**]. The [^{18}F] FDG-PET/CT findings were correlated with the NLR in all studied phases of COVID-19 disease. The persistence over time of increased [^{18}F] FDG uptake intensity may reflect a more severe acute phase of the disease.

The lung appears to be the most frequently involved organ in COVID-19, with reports of diffuse alveolar epithelium destruction, capillary damage/bleeding, hyaline membrane formation, alveolar septal fibrous proliferation, and/or pulmonary consolidation, among others [**12**,**24**]. Long-term follow-up studies of survivors of other coronavirus infections found that respiratory function limitations frequently last for months or even years, including impaired DLCO (in 15.5–43.6% of patients) and decreased TLC (5.2–10.9%) [**28**,**29**,**30**]. Various authors have addressed short- and medium-term respiratory function outcomes in survivors of COVID-19 infection, usually at hospital discharge [**31**,**32**]. In a study at 2–3 months post-discharge of 55 COVID-19 survivors who had not required mechanical ventilation, Zhao et al. described residual pulmonary function in 14 patients (25.45%), mainly impaired DLCO (in 13.6%) [**33**]. In a study at 6 weeks post-discharge of 124 COVID-19 survivors, van den Borst et al. [**34**] described an improvement in radiological images for almost all patients (99%) but observed residual lung parenchymal alterations in 91% of the patients and reduced lung diffusion capacity in 42%. Likewise, in their study at 3 months post-discharge of 76 healthcare workers who recovered from COVID-19, Liang et al. reported normal FEV1, FVC, FEV1/FVC, TLC, and DLCO values (>80% predicted) in 82% of the patients but the persistence of mild pulmonary function abnormalities in 42% [**35**]. The proportion of the present patients with impaired pulmonary function at 2–3 months was in line with previous findings on the short- to medium-term effects of COVID-19 infection [**33**,**34**].

The most frequent respiratory sequela of COVID-19 was DLCO alteration, as reported in previous studies, which may indicate the presence of pulmonary fibrosis [**12**,**24**]. DLCO and other respiratory function parameters were negatively correlated with the lung [^{18}F] FDG uptake as quantified by TLG. Although only a small proportion of the present patients had severe airway dysfunction, the results suggest that COVID-19 produces diffuse pulmonary epithelial damage and mild congestion of the airway mediated by lymphocyte-dominated interstitial inflammatory infiltrates. No published data appear to be available on the

association between respiratory function test results and pulmonary TLG. The majority of the present patients showed no lung lesions on CT scans at 2–3 months after discharge; however, pulmonary function was impaired in more than half of the patients with a normal lung CT scan. Hence, pulmonary function and [^{18}F]FDG-PET/CT testing is more sensitive than CT alone for identifying candidates for pulmonary rehabilitation after SARS-CoV-2 pneumonia [**22**].

High [^{18}F]FDG uptake may be related to increased anaerobic glycolysis caused by a cascade of reactions involving inflammatory cells [**7**,**36**]. In this way, the uptake of [^{18}F]FDG by lung lesions and lymph nodes observed in this study may be due to nonspecific immune or inflammatory activation, similar to the high [^{18}F]FDG uptake observed in lung lesions caused by the Middle East respiratory syndrome, pandemic H1N1 influenza virus, and organized pneumonia [**18**,**19**,**37**].

The [^{18}F] FDG-PET/CT offers a complementary approach to other imaging modalities by providing metabolic information. Although not currently recommended for the diagnosis of COVID-19 in the acute phase [**8**], it can yield relevant information for the diagnosis of short- and medium-term complications, including the chronic damage to the lungs and extrapulmonary sites that can follow acute infection [**6**,**22**]. However, radiologists and nuclear physicians need to develop a thorough understanding of the cellular mechanisms that underlie the pathophysiology of COVID-19 in the clinical settings of lung and extrapulmonary malignancies and inflammatory diseases in order to avoid misinterpretation of [^{18}F]FDG-PET/CT images [**31**].

Besides the small sample size, the main limitation of this study was the absence of a control group, hampering the possibility to detect causal relationships between the findings and COVID-19 infection. The epidemiological environment in which this study was carried out determined strict, restrictive conditions for access to hospital centers in our center and population. Evidently, the performance of [^{18}F] PET/CT in healthy collaborating patients was obviously not authorized. In addition, no test results were available for the baseline respiratory function of patients before COVID-19, although the presence of chronic lung disease was an exclusion criterion. Further research is required to fully elucidate the impact of COVID-19 on pulmonary function. In this regard, the present results cannot be extrapolated to patients with chronic lung disease. Another study limitation was the absence of a follow-up period to explore the long-term clinical relevance of the respiratory function impairment. Finally, biopsy specimens were not available for the studied organs. Nevertheless, the present findings contribute to laying the foundations for future studies with larger series on the potential role of [^{18}F] FDG-PET/CT in evaluating the sequelae of

COVID-19 infection. These should have prolonged follow-up periods to explore the possible relationship between initial lung inflammation and long-term sequelae such as residual lung fibrosis and respiratory failure.

5. Conclusions

In conclusion, at 2–3 months after the acute phase of SARS-CoV-2 infection, almost half of the patients evidenced an impairment of pulmonary function that was correlated with [^{18}F] FDG-PET/CT findings. In addition, the increased metabolic activity observed in the lung and mediastinal lymph node was associated with clinical and laboratory markers of disease severity. The [^{18}F] FDG-PET/CT is useful to obtain novel information on the pathogenesis of COVID-19 and on the diagnostic and evaluation of short- and medium-term sequelae, contributing to their management.

Author Contributions

E.M.T.-I., T.R.-S. and B.M.J.-R. contributed to collecting data, manuscript preparation/editing, and literature research; E.M.T.-I., B.M.J.-R., E.Y.G.-R., A.R.-F., J.M.L.-E., T.R.-S., M.G.-R. and C.M.-G. contributed to the study concept and design; E.M.T.-I. and B.M.J.-R. contributed to data analysis and interpretation and for manuscript editing; M.G.-R., J.M.L.-E. and C.M.-G. contributed to study design/planning and final approval. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Hospital Universitario Virgen de las Nieves (protocol code 1444-N-21).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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Conflicts of Interest

All authors declare that they do not have conflicts of financial or non-financial interest. Written consent was obtained for all patients and a local ethics committee approved the study.

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7.3. Artículo 3. Evidencias de calidad.

ABNORMAL ALPHA-1 ANTITRYPSIN LEVELS AND OTHER RISK FACTORS ASSOCIATED WITH LUNG FUNCTION IMPAIRMENT AT 6 AND 12 MONTHS AFTER HOSPITALIZATION DUE TO COVID-19: A COHORT STUDY.

(Niveles anormales de alfa 1 antitripsina y otros factores de riesgo asociados al deterioro de la función pulmonar a los 6 y 12 meses de la hospitalización por COVID-19: estudio de cohortes).

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Abnormal Alpha-1 Antitrypsin Levels and Other Risk Factors Associated with Lung Function Impairment at 6 and 12 Months after Hospitalization Due to COVID-19: A Cohort Study.

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Abstract: Respiratory function deficits are common sequelae for COVID-19. In this study, we aimed to identify the medical conditions that may influence lung function impairment at 12 months after SARS-CoV2 infection and to analyze the role of alpha-1 antytripsin (AAT) deficiciency (AATD). A cohort study was conducted on hospitalized COVID-19 pneumonia patients in Granada (Spain) during the first infection wave who were referred to a post-COVID-19 hospital clinic. The patients were monitored with three follow-up visits from May 2020 to May 2021. Previous medical history, hospital admission data, baseline parameters and physical examination data were collected at the first visit. Pulmonary function tests were performed at 6 and 12 months together with the determination of AAT level and

AATD genotype. After 12 months, 49 out of 157 patients (31.2%) continued to have lung function impairment. A multivariate analysis showed a statistically significant association of lung function impairment with: higher Charlson index; pneumonia with a central and/or
mixed distribution; anemia on admission; time in intensive care; need for corticosteroid boluses; abnormal respiratory sounds at 6 months; elevated lactate dehydrogenase at 12 months; abnormal AAT; and MZ genotype. Our results suggest that these medical conditions predispose COVID-19 patients to develop long-term lung function sequelae.

Keywords: COVID-19; respiratory function tests; alpha-1 antitrypsin; long-term follow-up.

1. Introduction

The term "Post-COVID-19 conditions" defined by the World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) is currently used to refer to continuous or new persistent symptoms, secondary sequelae from organ damage and effects of the disease or hospitalization that appear after the acute infection caused by Severe Acute Respiratory Syndrome (SARS) coronavirus 2 (SARS-CoV-2) [1-2]. Since other systemic viral diseases [3] and epidemics caused by previous coronaviruses (e.g., SARS-CoV-1, Middle East Respiratory Syndrome coronavirus [MERS]) have been associated with post-infectious sequelae and long-term complications [4,5], it was expected that they would also develop in post-COVID-19 patients. In this sense, alterations in respiratory function secondary to COVID-19 infection have been described, but they are not always related to the severity of the disease [6,7].

The CDC proposed α1-antitrypsin (AAT) deficiency (AATD) as a possible medical condition deserving further study due to mixed levels of evidence [8]. AAT is a glycoprotein belonging to the serpin group whose main function is to inhibit neutrophil elastase and prevent excessive proteolytic degradation of the connective tissue of the lungs [9,10]. AATD is a rare genetic disease due to mutations of the *SERPINA1* gene that produce low levels or defective AAT in the blood. This increases the risk of developing a variety of diseases, including pulmonary emphysema. Recent studies have described how this protein has biological functions that can counteract both SARS-CoV-2 infection and the underlying pathophysiological processes. It has also been suggested that patients with genotypic alterations have a higher risk of severity and even death [11,12]. In addition, a 2004 analysis of previous epidemics with other coronaviruses showed that serum samples from SARS patients had dramatically elevated levels of truncated forms of AAT, which correlated with the severity of SARS and indicated that these truncated forms of AAT could serve as SARS biomarkers with 100% sensitivity [13].

With the above in mind, the primary objective of the present study was to identify which medical conditions may influence the development of impaired lung function 12 month after COVID-19 infection. The secondary objective was to analyze the role of AATD and its related genetic mutations in such functional alterations.

2. Material and Methods

2.1. Study Design

This was a prospective, cohort study that included patients admitted to hospital for COVID-19 pneumonia from February to May 2020 (i.e., non-vaccinated patients from the "first wave" of COVID-19 in Spain) who were referred for follow-up to a post-COVID-19 respiratory clinic at the Virgen de las Nieves University Hospital in Granada, Spain. The patients were followed from May 2020 to May 2021. All were enrolled consecutively, and three follow-up visits were scheduled (**Figure 1**).

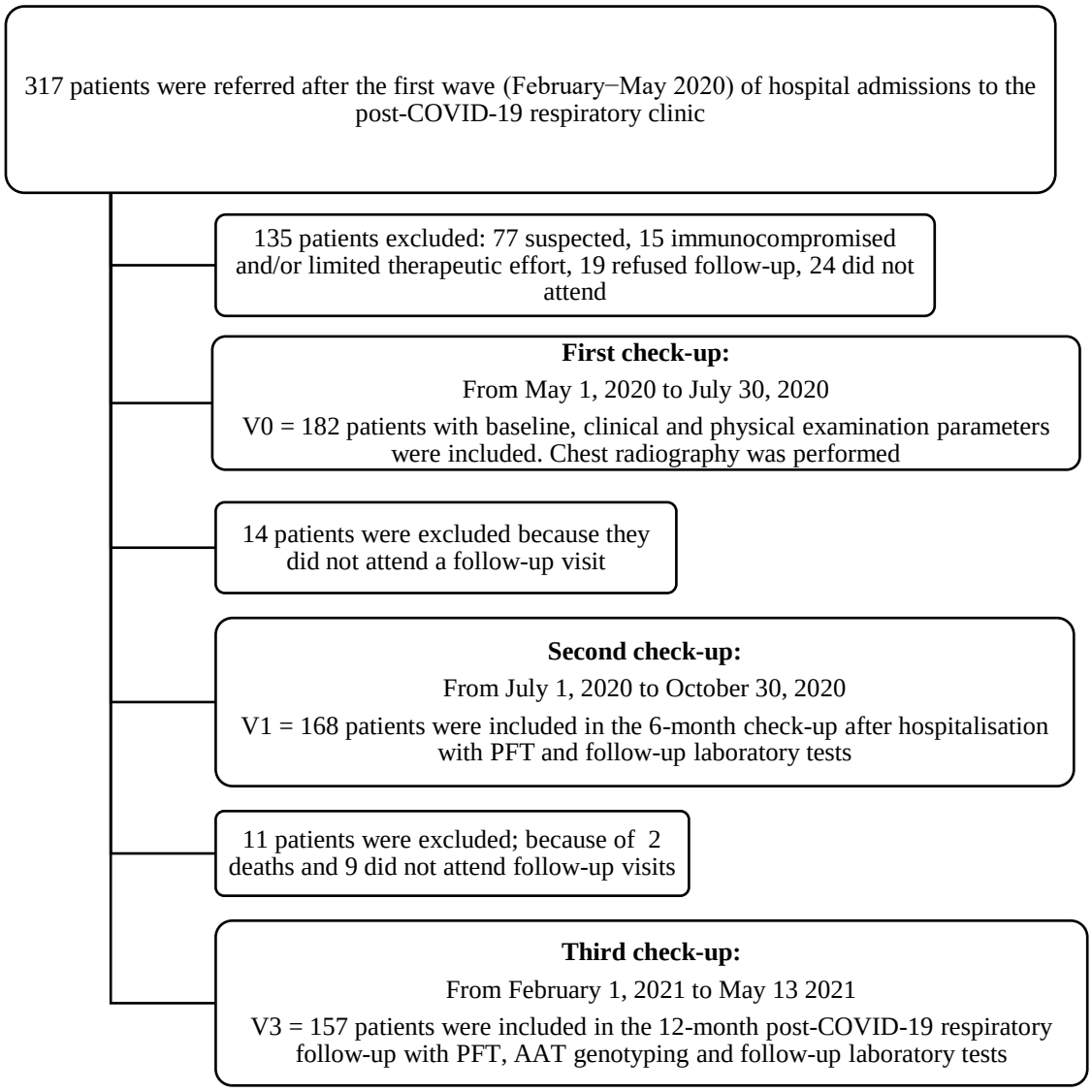

Figure 1. Patient flow chart after acute COVID-19 respiratory infection with first, second and third follow-up visits carried out at the University Hospital Virgen de las Nieves.

For COVID-19 diagnosis, RT-PCR from upper respiratory tract samples (nasopharyngeal or oropharyngeal swab) or lower respiratory tract samples (sputum collection) with antibody serology (IgM and IgG) by ELISA were used. The patients were followed for one year after the acute infection.

The first follow-up visit was two months after discharge from hospital. At that visit, previous medical history, characteristics of the hospital admission, baseline parameters and physical data were collected, and an X-ray was performed. The second follow-up was six months after discharge. Pulmonary function tests (PFT) and a 6-min walk test (6MWT) were performed. The last follow-up was carried out 12 months after hospital discharge and included laboratory tests and AAT levels along with genotyping and repeat PFT for those who had any abnormality in previous tests.

The study was carried out in accordance with the requirements stipulated in the Declaration of Helsinki (2013 revision) and Spanish Organic Law 15/1999 on the Protection of Personal Data. The study was approved by the Hospital's Ethics Committee, assigning it the internal code 1017-N-20.

2.2. Study Population

The inclusion criteria were: patients over 18 years of age with a confirmed diagnosis of SARS-CoV-2 infection (according to international recommendations [14]), hospitalization for COVID-19 pneumonia and informed consent. Exclusion criteria were: patients without confirmation of SARS-CoV2 infection, patients with underlying immunosuppression and patients in whom therapeutic efforts were limited.

2.3. Study Variables

2.3.1. During Hospitalization

Data were collected on demographics, medical history and characteristics of the hospitalization, especially the laboratory parameters related to the severity or mortality of the disease [15] (**Table S1 in the supplementary material**).

During the patients' hospital stay, chest X-rays in posterior–anterior and lateral projections were performed at diagnosis. Results were reported according to the recommendations of the Sociedad Española de Radiología Médica (SERAM) [Spanish Society of Medical Radiology], the international standard nomenclature defined by the Fleischner Society glossary, and available publications at the time of reporting [16,17]. The features of each X-ray performed were described in terms of density (alveolar, ground glass or mixed),

distribution (central, peripheral or diffuse), location (unilateral or bilateral) and extension (unilobar or multilobar).

2.3.2. During follow-up

At the 6-month follow-up, PFT with spirometric measurements, lung volumes and carbon monoxide diffusing capacity and a 6-min walk test (6MWT) were scheduled. The functional examination was carried out by experienced personnel using Jaeger MasterScreen Body whole body plethysmograph equipment (CareFusion Germany 234 GmbH, Hoechberg, Germany). Reference values and acceptability criteria were based on European and Spanish standards [18,19].

At the 12-month follow-up visit, further PFT were performed only on patients with functional alterations at the previous check-up. Plasma concentrations of AAT and C-reactive protein (CRP) were determined in all patients to rule out temporary elevation of AAT levels due to inflammation [20]. Genotyping of AAT was performed from a mouth swab using the Progenika A1AT Genotyping Test (Progenika Biopharma, a Grifols Company, Derio, Spain). The test allows simultaneous analysis of up to 384 samples per batch and is able to identify the 14 most frequent deficiency variants of the *SERPINA1* gene; details are available elsewhere [21].

The Progenika clinical service laboratory sequenced the *SERPINA1* gene if they did not find any of the 14 mutations and the serum AAT level was <60 mg/dL, or at the request of the treating physician. Sequencing of the seven exons of the gene was performed using latest generation-NGS techniques (MiSeq, Illumina Inc., San Diego, CA, USA); details are available elsewhere [21].

2.4. Statistical Analysis

In the descriptive analyses, continuous variables are given as mean and standard deviation. Categorical variables are given as numbers and percentages. For the comparison of quantitative data between the two groups for PFT variables altered at 12 months and abnormal AAT values, Student's t test for parametric independent data and the Mann–Whitney U test for non-parametric data were used. For quantitative data comparisons between qualitative variables with three or more groups, ANOVA was applied with the Bonferroni correction for multiple comparisons in the case of normality and the Kruskal–Wallis test otherwise. The association between the categorical variables was assessed by means of contingency tables applying the chi-square test for individual comparisons or Fisher's exact test for multiple comparisons. Univariate binary logistic regression analysis was

used to study the association between the different variables related to baseline comorbidities, demographic, clinical and physical characteristics and laboratory tests and functional respiratory recovery at 12 months after hospital discharge. Finally, a multivariate model was adjusted from those variables that showed a significant association in the univariate analysis. Data were processed for analysis using IBM SPSS Version 25.0 (IBM Corp, Armonk, New York, NY, USA) and/or mathematical computing R software. A p value < 0.05 was considered statistically significant.

3. Results

Out of 317 patients screened, a total of 182 patients hospitalised for SARS-CoV-2 infection were referred to post-COVID-19 respiratory clinics for review. The follow-up study lasted for one year after the acute infection with 168 patients (92.3%) attending the 6-month check-up and 157 (86.3%) the 12-month check-up (**Figure 1**).

The clinical characteristics of the study population separated according to normal or impaired lung function at 12 months are shown in **Table 1**. Details of laboratory parameters are provided in **Table S2 of the supplementary material**.

Table 1. Baseline comorbidities, demographics, clinical and physical characteristics of the patients at admission, during hospitalization and at the 12-month follow-up visit.

Baseline Characteristics	**Lung Function at 12 Months [Mean ± SD or n (%)]**		***p*-Value**
	Normal (n = 108)	**Impaired (n = 49)**	
Age	57.4 ± 12.5	62.4 ± 13.0	0.026
Male	68 (63)	25 (51)	0.272
Smoking history:			
Active smoker	6 (5.6)	3 (6.1)	0.201
Never smoked	64 (59.3)	25 (51)	
Former smoker	38 (35.2)	21 (35.6)	
Cumulative smoking burden index (smokers and former smokers)	11.3 ± 19.9	14.8 ± 24.9	0.039
BMI (kg/m^2)	29.4 ± 5.0	29.1 ± 5.0	0.938
Previous medical history			
≥2 baseline comorbidities	37 (34.3)	31 (63.3)	0.006
HBP	45 (41.7)	25 (51)	0.413
DM2	10 (9.3)	13 (26.5)	0.006
Dyslipidemia	23 (21.5)	14 (28.6)	0.599
Coronary heart disease	3 (2.8)	1 (2)	0.075
Cardiac arrhythmias	4 (3.7)	4 (8.2)	0.715
Cerebrovascular disease	2 (1.9)	3 (6.1)	0.391
Baseline respiratory comorbidities	22 (20.4)	20 (40.8)	0.008
COPD	3 (2.8)	8 (16.3)	0.006
Baseline liver disease	3 (2.8)	2 (4.1)	0.638
Chronic kidney disease	6 (5.6)	4 (8.2)	0.633
Active cancer	1 (2%)	1 (0.6%)	0.401
Previous cancer history < 5 years	5 (4.7)	5 (10.2)	0.526
Charlson index ≥ 3	32 (29.6)	32 (65.3)	0.002
Signs and symptoms on admission			

Fever (>37.5 °C)	103 (72.5)	39 (79.6)	0.002
Dyspnoea	62 (57.9)	39 (79.6)	0.010
Cough	79 (73.8)	38 (77.6)	0.638
Exhaustion	83 (77.6)	31 (63.3)	0.064
Musculoskeletal pain	60 (56.1)	17 (35.4)	0.019
Anosmia/ageusia	24 (22.4)	13 (26.5)	0.449
Gastrointestinal symptoms	27 (64.3)	15 (30.6)	0.441
Cardiac symptoms	4 (3.7)	3 (6.1)	0.441
Neurological symptoms	16 (15)	8 (16.3)	0.754
Dermatological symptoms	1 (0.9)	1 (2.0)	0.270
ARDS (PaO2/FiO2 < 300 mmHg)	21 (19.6)	19 (38.8)	0.012
Hospitalization			
Mean length of stay (days)	9.9 ± 11.2	14.8 ± 14.0	0.043
Pneumonia on admission	101 (93.5)	41 (83.7)	0.047
Characteristics of pneumonia on chest X-ray (n = 142)			
-Density:	25 (24.8)	13 (31.7)	0.247
• Alveolar	19 (18.8)	5 (12.2)	
• Ground-glass opacity (GGO)	57 (56.4)	23 (56.1)	
• Mixed			
-Distribution:			
• Central	6 (5.9)	8 (19.5)	0.047
• Peripheral	68 (67.3)	17 (41.5)	
• Mixed	27 (26.7)	16 (39)	
-Location:			
• Unilateral	10 (9.9)	5 (12.2)	0.505
• Bilateral	91 (90.1)	36 (87.8)	
-Involvement:			
• Single lobe	9 (8.9)	3 (7.3)	0.827
• Multilobar	92 (91.1)	38 (92.7)	
Admission to the ICU	3 (2.8)	6 (12.2)	0.030

Endotracheal intubation (ETI)	2 (1.9)	6 (12.2)	0.017
Treatment received:	39 (58.2)	28 (41.8)	0.019
• Corticosteroids (prednisone, methyprednisolone)	92 (68.1)	43 (31.9)	0.625
• Antivirals	7 (43.8)	9 (56.3)	0.031
• Tocilizumab	3 (75)	1 (25)	0.679
• Anakinra	102 (69.4)	45 (30.6)	0.182
• Antimalarials (hydroxychloroquine)			

The results are expressed as frequency (%) and mean (±SD). Abbreviations: BMI = body mass index, ARDS = acute respiratory distress syndrome, IL-6 = interleukin 6; COPD = chronic obstructive pulmonary disease; DM2 = diabetes mellitus-2; HBP = high blood pressure; ICU = intensive care unit; ETI = endotracheal intubation.

The study population was predominantly males (93 [59.2%]) with a mean age of 59.9 years (±12.9) and a mean BMI of 29.4 (±5.0). Ninety-eight patients (62.4%) were active smokers or former smokers with a mean cumulative smoking burden index (ICAT) of 12.4 (±21.6) pack-years. A total of 68 patients (43.3%) had two or more baseline comorbidities with a Charlson index of 3 or higher in 64 patients (40.8%). The mean hospital stay was 11.5 days (±2.8), and the most common clinical manifestations while in hospital (affecting over 25% of patients) were fever, dyspnoea, cough, fatigue, musculoskeletal pain and gastrointestinal symptoms (The admission chest X-ray showed pneumonia in 142 cases (90.4%). Sixty-seven patients (43.2%) received corticosteroids (prednisone and/or methylprednisolone) and 135 (87.1%) received antiretrovirals during treatment.

3.1. Association between the Patients Clinical Characteristics and Lung Function 12 Months after Hospital Discharge

Of the total of 157 patients who completed the study, 49 (31.2%) met the criteria for abnormal PFT at 12 months, i.e., they had impaired lung function. The remaining 108 patients (68.8%) had PFT within normal limits.

The comparison of the two groups (abnormal vs. normal PFT) using univariate binary logistic regression analysis is shown in **Table 2**.

Table 2. Risk factors associated with impaired lung function at 12 months for the bivariate and multivariate analysis.

Covariate	OR[a]	*p*-Value	95% CI for OR	Explained Variance, R^2
(A) Univariate logistic regression analysis				
Age	1.032	0.026	1.004–1.061	0.046
Charlson index	1.421	<0.001	1.186–1.703	0.139
≥2 comorbidities	3.305	0.001	1.635–6.680	0.100
DM2	3.539	0.006	1.426–8.779	0.065
Heart disease	2.833	0.075	0.899–8.931	0.028
Lung disease	2.696	0.008	1.290–5.636	0.061
COPD	6.829	0.006	1.727–27.013	0.075
Signs and symptoms on admission				
Fever	0.151	0.002	0.045–0.511	0.091
Dyspnoea	2.831	0.010	1.280–6.260	0.064
Asthenia	0.498	0.064	0.238–1.041	0.030
Myalgia	0.430	0.019	0.212–0.869	0.051
X-ray distribution				
Peripheral	Ref			0.095
Central	5.333	0.006	1.632–17.434	
Mixed	2.370	0.038	1.049–5.357	
Haemoglobin (g/dL)	0.780	0.017	0.636–0.956	0.054
Leucocytes (count $\times 10^3/\mu L$)	1.172	0.011	1.037–1.324	0.061
Neutrophils (count $\times 10^3/\mu L$)	1.175	0.024	1.022–1.352	0.051
Lymphocytes (%)	0.957	0.044	0.916–0.999	0.041
NLR	1.144	0.006	1.040–1.259	0.080
Platelets (count $\times 10^3/\mu L$)	1.004	0.012	1.001–1.008	0.061
Hospitalization				

ICU admission	4.884	0.030	1.168–20.420	0.045
ETI	7.395	0.017	1.436–38.089	0.060
ARDS	2.594	0.012	1.229–5.474	0.055
Corticosteroid therapy	2.291	0.019	1.149–4.566	0.050
Corticosteroid boluses	3.665	<0.001	1.765–7.612	0.107
Tocilizumab	3.182	0.031	1.109–9.128	0.041
Haemoglobin (g/dL)	0.759	0.008	0.620–0.930	0.076
Albumin (g/dL)	0.439	0.048	0.195–0.991	0.043
LDH (U/L)	1.004	0.011	1.001–1.007	0.065
Procalcitonin	10.160	0.052	0.982–105.105	0.062
IL-6 (pg/mL)	1.107	0.090	0.997–1.036	0.120
LTOT	4.921	0.003	1.701–14.235	0.080
Length of hospital stay (days)	1.003	0.043	1.001–1.066	0.045
		Follow-up		
Haemoglobin (g/dL)	0.763	0.012	0.619–0.941	0.061
Platelets (count $\times 10^3/\mu L$)	1.005	0.028	1.001–1.010	0.051
LDH (U/L)	1.011	0.017	1.002–1.021	0.063
Ferritin (ng/mL)	0.995	0.023	0.991–0.999	0.065
Troponin (ng/L)	0.782	0.007	0.654–0.934	0.100
Vitamin D (ng/mL)	0.955	0.058	0.910–1.002	0.042
Abnormal respiratory sounds	7.186	0.018	1.395–37.023	0.059
(B) Multivariate logistic regression analysis				
Charlson index	1.336	0.030	1.029–1.735	
X-ray distribution				
Peripheral	Ref			0.534
Central	10.820	0.004	2.093–55.934	
Mixed	4.855	0.014	1.374–17.154	

Admission haemoglobin (g/dL)	0.604	0.006	0.422–0.864
ICU admission	33.184	0.012	2.180–505.072
Methylprednisolone boluses	3.447	0.043	1.039–11.433
Follow-up LDH (U/L)	1.025	0.004	1.008–1.042
Abnormal respiratory sounds	15.157	0.027	1.011–227.244

OR = odds ratio; Ref = reference category; LDH = lactate dehydrogenase; NRL = *neutrophil/lymphocyte ratio; IL-6 = interleukin 6; LTOT = long-term oxygen therapy. ICU = intensive care unit; ETI = endotracheal intubation; ARDS = acute respiratory distress syndrome; DM2 = Diabetes mellitus type-2. (a)* The OR account for a 1 unit increase in each of the independent variables.

After univariate analysis of the association of the different variables with the risk of having impaired lung function, a multivariate model was adjusted, which initially contained all the variables. From this model, a predictive model was adjusted that included the following as predictors of alterations of lung function 12 months after hospitalization: the Charlson index; pneumonia with a mixed and/or central distribution; anaemia on admission; admission to the ICU; need for treatment with corticosteroid boluses; persistence of abnormal respiratory sounds at the 6-month check-up; and lactate dehydrogenase (LDH) elevation at the 12-month follow-up (**Table 2**).

3.2. Lung Function at 6 and 12 Months after Hospitalization

All patients who attended the 6-month follow-up were asked to undergo PFT and a 6MWT, but only 150 completed these tests. Of that 150, 67 (42.7%) had some type of functional impairment. The same patients were asked to undergo repeat PFT at the 12-month check-up, and 49 patients (31.2%) continued to have impaired lung function. Therefore, 18 patients (11.5%) with abnormal PFT at 6 months had recovered functionally at 12 months (**Figure 2**). The serial values for the PFT at 6 and 12 months post discharge in the patients who had pulmonary functional alterations are shown in **Table 3**.

Table 3. Serial values of pulmonary function tests at 6 and 12 months in the subgroup of patients with impaired lung function. Data are expressed as percentage (%) or mean ± standard deviation (SD).

Respiratory Function Parameters	At 6 Month (n = 67) (Mean ± SD)	At 12 Month (n = 49) (Mean ± SD)	Difference (Mean ± SD)	95% CI	*p*-Value
FVC (%)	91.2 ± 17.5	92.2 ± 19.7	−0.98 ± 12.4	−4.340–2.369	0.558
FEV1 (%)	87.1 ± 20.3	90.8 ± 21.6	−3.6 ± 16.0	−7.958–0.708	0.099
FEV1/FVC	75.0 ± 11.5	77.4 ± 10.2	−2.4 ± 9.4	−4.909–0.176	0.067
TLC (%)	98.0 ± 18.4	102.6 ± 21.9	−4.6 ± 14.6	−8.671–0.555	0.027
DLCO (%)	73.6 ± 19.0	76.6 ± 13.5	−2.9 ± 17.6	−7.925–2.069	0.245
KCO (%)	92.3 ± 20.8	95.1 ± 16.1	−2.8 ± 17.9	−7.866–2.282	0.274
RV (%)	100.4 ± 30.8	105.6 ± 47.0	−5.3 ± 38.4	−15.96–5.41	0.327
Distance 6MWT (m)	466.4 ± 107.9	430.0 ± 114.7	36.4 ± 79.9	12.95–59.89	0.003
Initial SaO2 6MWT %)	96.5 ± 1.8	95.8 ± 2.4	0.71 ± 2.6	−0.040–1.456	0.063
Final SaO2 6MWT %)	93.7 ± 4.0	92.7 ± 4.2	1.0 ± 3.3	0.000–1.917	0.05
Initial—final SaO2 6MWT (%)	2.9 ± 4.2	3.2 ± 4.3	−0.25 ± 4.1	−1.452–0.952	0.677

FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; TLC = total lung capacity; RV = residual volume; DLCO = diffusing capacity for carbon monoxide; 6MWT = six minute walk test; SaO2 = oxygen saturation.

Figure 2. Evolution of pulmonary function tests at 6 and 12 months follow-up. Data are expressed as percentege (%). Abbreviations: FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; TLC = total lung capacity; RV = residual volume; DLCO = carbon monoxide transfer by single breath; KCO= diffusion constant for carbon monoxide; VR = residual volume; 6MWT = six-minute walking test; $SatO_2$ = oxygen saturation.

3.3. Association between AAT Levels and Genotyping and Functional Recovery

The data from the 157 patients who attended the third follow-up were categorised according to genotype with the corresponding plasma levels of AAT and elevated CRP value > 5 mg/dL (**Table S3, supplementary material**); 83.4% of hospitalised patients had normal AAT genotype (Pi*MM), with plasma AAT levels of 128.6 ± 1.4 mg/dL. Other genotypic variants found were Pi*MS (14.6%), Pi*MZ (1.3%) and Pi*M/P Lowell (0.6%) with plasma AAT levels of 116.4 ± 3.2, 89.0 ± 15.0 and 114.8 ± 0.0 mg/dL, respectively.

Figure 3 shows a graph of the mean plasma levels of AAT for each category of AAT genotype analysed with patients stratified based on the CRP level (categorised as normal if ≤5 mg/L or elevated if >5 mg/L) for all hospitalised patients and for the subgroups with normal or impaired lung function at 12 months.

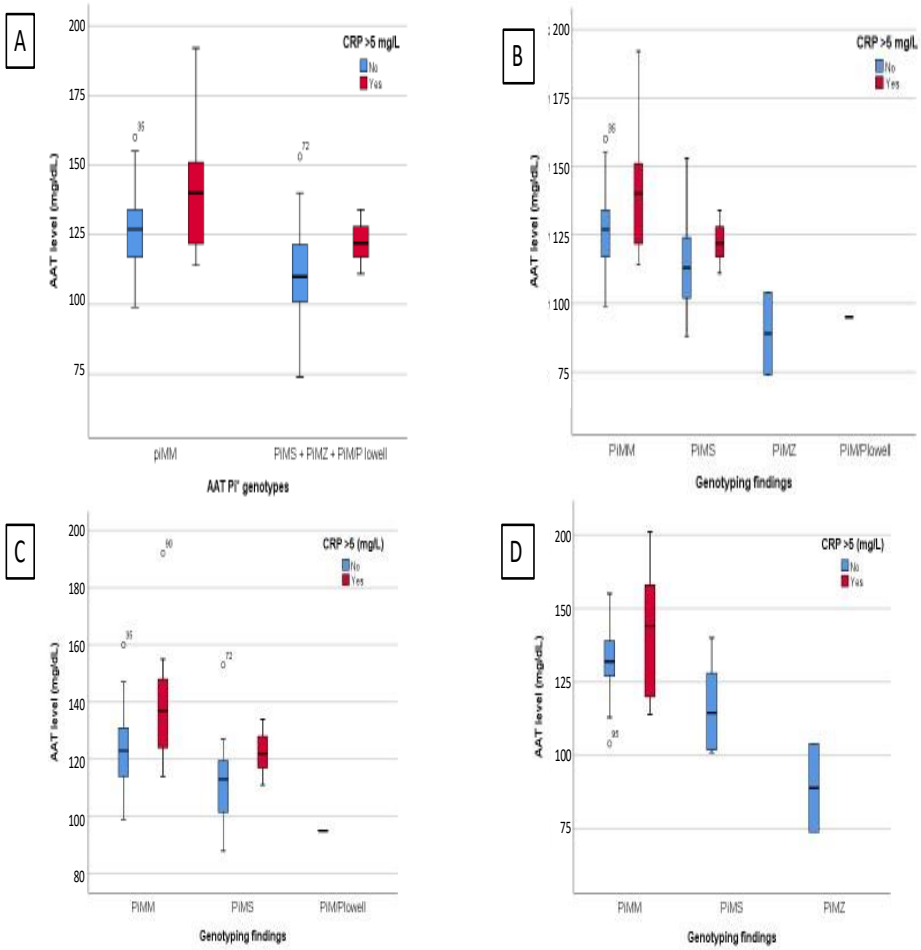

Figure 3. Findings from the plasma determination of AAT, allelic genotype and CRP determination for all hospitalised patients and for the subgroups of patients with normal or impaired lung function at 12 months. (**A**) Breakdown of AAT levels classified by normal genotype (Pi*MM) or unified heterozygous deficiencies (Pi*MS, Pi*MZ and Pi*M/P Lowell) and stratified by normal or elevated CRP for all hospitalised patients. (**B**) Comparison of AAT levels based on genotyping (Pi*MM, Pi*MS, Pi*MZ and Pi*M/P Lowell) and the normal or high CRP value for hospitalised patients. C and D) AAT values analyzed based on genotype and normal or elevated CRP of the subgroups of patients with normal (**C**) or impaired lung function (**D**) at the 12 month follow-up.

At 6 months, the functional parameters of forced expiratory volume in the first second (FEV1) (69.9 ± 22.8 vs. 99.9 ± 17.8; p = 0.02), FEV1/forced vital capacity (FVC) (66.4 ± 13.6 vs. 78.4 ± 7.0, p = 0.018) and total lung capacity (TLC) (79.5 ± 19.1 vs. 102.9 ± 14.8, p = 0.029) were significantly lower in the abnormal AAT group than in the normal AAT group. However, at 12 months, only TLC values (75.0 ± 12.2 vs. 104.9 ± 20.1, p = 0.041) showed a significant decrease in the abnormal AAT group.

For allele genotyping and respiratory function variables at 6 months, significant differences were found in the carbon monoxide transfer by a single breath (DLCO) (p = 0.045) and the distance covered in the 6MWT (p = 0.040). The analysis of multiple comparisons

(**Table S4 in the supplementary material**) showed differences between the groups with the Pi*MM and Pi*MZ genotypes for TLC (mean difference 28.7 ± 10.6; *p* = 0.023), DLCO (mean difference 41.96 ± 15.5; *p* = 0.024) and the distance covered in the 6MWT (mean difference 210.2 ± 74.9 m, *p* = 0.017).

Table 4 shows how normal AAT plasma levels (range 83–220 mg/dL) or abnormal levels, along with the allelic genotype detected (Pi*MM, Pi*MS, Pi*MZ and Pi*M/P Lowell), influence the recovery of respiratory function variables at 6 and 12 months of follow-up.

Table 4. Association between alpha-1 antitrypsin (AAT) levels results and allelic genotype with pulmonary function test parameters at 6- and 12-month follow-up visits.

	AAT Levels (mg/dL)			**Genotype**				
	Normal	**Abnormal**	***p*-Value**	**Pi*MM**	**Pi*MS**	**Pi*MZ**	**Pi*MP Lowell**	***p*-Value**
(A) Abnormal PFT (mean ± SD) at 6 months (N = 66)								
FEV1 %	99.9 ± 17.8	69.9 ± 22.8	0.020	100.4 ± 18.7	92.1 ± 17.2	78.9 ± 35.4	114.8	0.086
FVC %	106.7 ± 76.0	79.7 ± 10.9	0.618	101.0 ± 16.4	134.0 ± 183.9	86.3 ± 20.2	113.7	0.244
FEV1/FVC %	78.4 ± 7.0	66.4 ± 13.6	0.018	78.0 ± 8.1	77.0 ± 9.1	69.8 ± 18.5	79.1	0.549
TLC %	102.9 ± 14.8	79.5 ± 19.1	0.029	103.5 ± 14.7	102.6 ± 16.3	74.9 ± 12.5	107.8	0.065
DLCO %	88.6 ± 22.1	80.4 ± 40.7	0.607	89.5 ± 22.2	85.2 ± 19.8	47.7 ± 5.6	103.4	0.045
KCO %	100.2 ± 17.7	110.0 ± 25.5	0.438	99.9 ± 18.0	100.1 ± 17.4	79.4 ± 17.8	109.4	0.414
RV %	102.1 ± 26.4	76.8 ± 22.9	0.181	100.4 ± 24.8	114.2 ± 36.1	69.5 ± 12.6	93.1	0.055
6MWT Distance (m)	507.0 ± 110.3	568.5 ± 115.3	0.436	516.0 ± 100.8	494.5 ± 115.1	306.0 ± 256.0	-	0.040
6MWT Initial SaO2 (%)	96.5 ± 1.5	95.5 ± 2.1	0.346	96.6 ± 1.6	96.6 ± 1.5	95.0 ± 2.8	-	0.567
6MWT Final SaO2 (%)	95.0 ± 3.8	91.5 ± 5.0	0.198	95.0 ± 3.9	95.0 ± 3.9	89.5 ± 2.1	-	0.224
(B) Abnormal PFT (mean ± SD) at 12 months (N = 49)								
FEV1 %	95.9 ± 20.4	69.4 ± 23.6	0.075	97.0 ± 19.7	94.1 ± 24.1	64.1 ± 16.6	-	0.083
FVC %	77.7 ± 9.2	64.9 ± 14.4	0.257	98.3 ± 19.5	95.6 ± 19.8	71.1 ± 2.8	-	0.149

FEV1/FVC %	96.9 ± 2.3	81.3 ± 8.2	0.059	77.6 ± 8.9	76.7 ± 13.0	70.8 ± 22.8	-	0.616
TLC %, SD	104.9 ± 20.1	75.0 ± 12.2	0.041	105.7 ± 20.5	100.6 ± 16.7	74.9 ± 12.2	-	0.087
DLCO %, SD	82.7 ± 15.1	75.5 ± 40.2	0.525	82.8 ± 15.5	81.1 ± 11.0	47.0	-	0.067
KCO %, SD	111.9 ± 118.7	101.6 ± 23.5	0.903	111.3 ± 117.1	98.0 ± 15.5	84.9	-	0.916
RV %, SD	108.7 ± 41.8	59.9 ± 5.9	0.105	109.8 ± 42.3	92.2 ± 4	66.5 ± 15.3	-	0.196
6MWT Distance (m)	442.8 ± 104.2	567.5 ± 24.7	0.098	443.3 ± 102.0	441.9 ± 107.0	550.0	-	0.588
6MWT Initial SaO2 (%)	95.7 ± 2.2	97.5 ± 2.1	0.258	95.7 ± 2.3	95.5 ± 1.9	99.0	-	0.329
6MWT Final SaO2 (%)	93.3 ± 3.9	94.5 ± 2.1	0.662	92.9 ± 4.0	94.6 ± 2.7	96.0	-	0.317

Abbreviations: SD = standard deviation FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; TLC = total lung capacity; RV = residual volume; DLCO = diffusing capacity for carbon monoxide transfer in single breath; 6MWT = six minute walk test; SaO2 = oxygen saturation; AAT= Alpha-1 antitrypsin; PFT= pulmonary function test.

4. Discussion

Published reports issued in the weeks or months after discharge from hospital for COVID-19 pneumonia describe patients with varying degrees of persistent symptoms and radiological and functional abnormalities [**22**,**23**]. In the present study, risk factors associated with impaired lung function in patients who were hospitalised for COVID-19 were identified: a higher Charlson index, severe pneumonia with anemia, need for corticosteroid boluses of methylprednisolone, admission to the ICU. Our results are in line with those published to date on risk factors associated with a worse clinical course, acute respiratory distress syndrome (ARDS) and death during hospitalization [**24**,**25**]. In this study, high levels of haemoglobin, lymphocytes, ferritin, albumin and troponin significantly reduced the risk of impaired lung function. The elevation of troponin and ferritin has previously been reported as a risk factor for poor clinical outcome, in contrast to our results. One explanation for their protective role may be the fact that these parameters increase early in cases with a poor clinical course and development of the inflammatory cascade thus potentially allowing early diagnosis and management.

We also found that close to one third of the patients (n = 49; 31.2%) continued to have impaired lung function at 12 months. The most affected functional parameters being FEV1 and DLCO. To date, there are studies that report on short- and medium-term functional outcomes [**22**,**26**]. However, studies evaluating one-year results have only recently become available. Wu et al. described persistent physiological (DLCO: 88% reduction of predicted) and radiographic (24% of patients) abnormalities in some patients 12 months after discharge for COVID-19 treatment [**26**]. Liu et al. reported physiological, laboratory, radiological or electrocardiogram abnormalities, with those related to renal, cardiovascular and liver function being particularly common, in patients who recovered from COVID-19 up to 12 months post-discharge [**27**]. Huang et al. reported 30% of patients with dyspnoea and 26% of patients with anxiety or depression at the 12-month visit among COVID-19 survivors [**28**]. In general for our study, all the functional variables improved from 6 to 12 months. However, only TLC and the distance covered in metres in the 6MWT reached statistical significance. Both variables showed a statistically significant decrease despite improvement in lung volumes. This may be due to persistent muscle weakness, which can cause dyspnoea on exertion and limit performance in this test.

With regard to AAT levels, two patients were in the abnormal range (both below normal), one with normal and one with impaired lung function. The mean level of AAT was similar in patients with normal and impaired lung function (124.4 ± 15.5 and 129.1 ± 18.2, respectively). However, there were 28 patients with normal levels of AAT and CRP > 5 mg/L. It is worth noting that AAT is an acute-phase reactant and its plasma levels have been shown to increase 2–3 times in response to inflammatory or infectious stimuli, similar to CRP [**20**]. Complete genome sequencing was requested for these patients, but there were no pathogenic variants found.

The allelic variants of AAT in our patients were distributed in a similar way to the general population [**29**]; Pi*MM being predominant (131 patients: 83.4%) and deficient genotypes being far less common (Pi*MS 22 patients: 14.6%, Pi*MZ 2 patients: 1.3% and Pi*M/P Lowell 1 patient: 0.6%). Significant differences between the groups with normal and impaired lung function at 12 months were seen only in the normal genotype (Pi*MM). However, it should be noted that the two Pi*MZ cases and half of the Pi*MS cases were in the group of patients with impaired lung function. Moreover, the analysis of multiple comparisons revealed that there were statistically significant differences between PiMM and PiMZ genotypes in the patients with normal pulmonary function regarding DLCO and TM6M. In other study, the most common mild AATD genotypes were associated neither with increased SARS-CoV-2

infection rates nor with increased SARS-CoV-2 fatalities. The numbers of patients with severe AATD cases were too low to allow definitive conclusions [**30**].

With this study being single-centre and observational, there is a potential location bias. However, our study does have several strengths. It has a large sample size and included patients with moderate or severe COVID-19 with previous hospitalization who continue to have long-term functional sequelae. Many characteristics that influenced their recovery had not previously been reported.

In conclusion, our study found that close to one third of COVID-19 patients still showed impaired lung function 12 months after infection. The presence of a high Charlson index, severe pneumonia with anaemia, need for corticosteroid boluses, admission to the ICU and low AAT levels or Pi*MZ deficiency allele variants predisposed patients to impaired lung function at 12 months after hospitalisation due to COVID-19.

Supplementary Materials

The following are available online at **https://www.mdpi.com/article/10.3390/healthcare10122341/s1**, Figure S1: Summary of the characteristics of the patients included in the study, Figure S2: Laboratory parameters of patients on admission, during hospitalization and at the 12-month follow-up, Figure S3: Number and proportion of hospitalized patients with normal or impaired lung function at 12 months with plasma determination of alpha-1 antitrypsin (AAT), allelic genotyping (Pi*MM, Pi*MS, Pi*MZ and Pi*MP Lowell) and C-reactive protein (CRP) > 5 mg/dL, Figure S4: Number and proportion of hospitalized patients with normal or impaired lung function at 12 months with plasma determination of alpha-1 antitrypsin (AAT), allelic genotyping (Pi*MM, Pi*MS, Pi*MZ and Pi*MP Lowell) and C-reactive protein (CRP) > 5 mg/dL.

Author Contributions

C.M.-G. designed and conducted the study with assistance from B.M.J.-R., J.G.-F. and E.M.T.-I.; B.M.J.-R. collected the demographic, clinical, psychological and pulmonary function test data in follow-up consultation supported by A.D.R.-O. and E.M.T.-I.; B.M.J.-R. captured and processed the HRCT images; J.G.-F. processed the laboratory indices; E.M.T.-I. and B.M.J.-R. analysed the data in SPSS; C.M.-G., B.M.J.-R., E.M.R.-U. and E.M.T.-I. wrote

the manuscript; and all authors contributed to the discussion and interpretation of the results. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was carried out in accordance with the requirements stipulated in the Declaration of Helsinki (Tokyo revision, October 2004) and Spanish Organic Law 15/1999 on the Protection of Personal Data. The study was approved by the Hospital's Ethics Committee, assigning it the internal code 1017-N-20.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest

The authors declare that they have no other competing interests.

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Supplementary material for

Abnormal Alpha-1 Antitrypsin Levels and Other Risk Factors Associated with Lung Function Impairment at 6 and 12 Months after Hospitalization Due to COVID-19: A Cohort Study.

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Table S1. Summary of the characteristics of the patients included in the study.

First follow-up consultations
Goes for consultation, N=182 patients
Characteristics
Age (years), median (IQR)
Sex, N (%):
Male
Female
BMI (kg/m2)
Smoking history, N° (%):
Cumulative tobacco burden index **(**ICAT**)**, N° (%):
Current
Former
Nonsmoker
Personal history.
Baseline comorbidities <2; ≥2, N° (%)
High blood pressure (HBP), N° (%)
Diabetes Mellitus 2 (DM2), N° (%)
Dyslipemia, N° (%)
Ischemic cardiopathy: N° (%)
Cardiac arrhythmias N° (%)
Cerebrovascular disease, N° (%)
Baseline respiratory comorbidities, N° (%)
Chronic obstructive pulmonary disease (COPD), N° (%)
Baseline liver disease, N° (%)
Chronic kidney disease, N° (%)
Active tumor, N° (%)
History of previous tumor <5 años, N° (%)
Charlson Comorbility index <3; ≥3, N° (%)
Admission symptoms
Fever (>37.5ºC), N° (%)
Dyspnea, N° (%)
Cough, N° (%)
Fatigue, N° (%)

Musculoskeletal discomfort, N° (%)
Anosmia/ageusia, N° (%)
Digestive symptoms, N° (%)
Cardiac symptoms, N° (%)
Neurological symptoms, N° (%)
Dermatological symptoms, N° (%)
ARDS (PaO2/FIO2 < 300 mmHg):
Hospitalization.
Average length of stay (days)
Pneumonia on admission No. (%)
• Characteristics of pneumonia on chest X-ray, No. (%) -Density: Alveolar Ground glass opacity (GGO) Mixed -Distribution: Central Peripheral Mixed -Location: Unilateral Bilateral -Extension: Unilobar Multilobar
ICU admission, No. (%)
Orotracheal intubation (OTI, No. (%)
Treatment received, No. (%) - Corticosteroids - Antiviral drugs - Tocilizumab - Anakinra - Antimalarials (hydroxychloroquine)
Second follow-up consultations
Goes for consultation, N= 168
Pulmonary function
FVC <80%, % of predicted
FEV1 <80%, % of predicted
FEV1/FVC <70%

TLC <80%, % of predicted
VR <65%, % of predicted
DLCO <80%, % of predicted
KCO<80%, % of predicted
6MWT
Distance-meters, median (IQR)
Oxygen saturation, median (IQR)
Average
Initial
Final
Third follow-up consultations
Goes for consultation, N=157
Pulmonary function performed only on patients with alterations at 6 months.
Laboratory findings at 12 months (mean ± SD)
Alpha 1 antitrypsin (mg/dl)
Alpha antitrypsin normal range, N° (%)

ARDS=Acute respiratory distress syndrome; ICU= Intensive care unit; BMI= body mass index; IOT=Intubation orotraqueal; FVC=forced vital capacity; FEV1=forced expiratory volume in the first second; TLC=total lung capacity; RV=residual volume; DLCO=carbon monoxide transfer by single breath; KCO= diffusion constant for carbon monoxide; 6MWT=six-minute walking test; SD: standard deviation.

Table S2. Laboratory parameters of patients on admission, during hospitalization and at the 12-month follow-up.

Laboratory parameters	Lung function at 12 months		p-value
	Normal (n=108) (mean ± SD) or n (%)	Impaired (n=49) (mean ± SD) or n (%)	
On admission			
Hemoglobin (g/dl)	14.5±1.5	13.7±2.1	0.017
Leukocytes (count x10^3/µl)	6.3±2.6	7.7±3.2	0.011
Neutrophils (count x10^3/µl)	4.8±2.4	5.9±2.7	0.024
Lymphocytes (count x10^3/µl)	1.3±1.1	1.1±0.5	0.044
Ferritin (ng/ml)	745.3±857.9	624.5±688.3	0.523
Platelets (count/µl)	214,819±85,918	263,851±135,238	0.012
C-Reactive Protein (mg/l)	79.2±68.8	88.7±73.1	0.358
Lactate dehydrogenase (U/l)	344.1±122.5	372.4±113.3	0.161
AST/Aspartate transaminase (U/l)	44.0±31.8	38.2±26.3	0.407
ALT/Alanine transaminase (U/l)	40.0±30.1	32.9±22.2	0.736
Albumin (g/dl)	4.0±0.8	5.1±6.9	0.360
D-dimer (mg/l)	0.76±1.07	2.3±5.4	0.084
Fibrinogen (mg/dl)	641.0±193.7	666.6±213.2	0.255
Blood glucose (mg/dl)	115.2±38.2	123.8±55.0	0.307
During hospitalization			
Hemoglobin (g/dl)	15.4±15.9	13.0±2.0	0.008
Leukocytes (count x10^3/µl)	7.6±3.7	8.0±3.1	0.466
Neutrophils (count x10^3/µl)	6.1±5.1	6.0±3.3	0.969
Lymphocytes (count x10^3/µl)	1.4±1.0	1.3±0.8	0.206
Ferritin (ng/ml)	1,133±1,560	1,577±2,043	0.197
Platelets (count/µl)	323,641±188,349	329,674±163,188	0.993
C-Reactive Protein (mg/l)	54.2±102.5	70.3±77.2	0.400
Lactate dehydrogenase (U/l)	328.8±104.3	386.6±145.2	0.011
AST/Aspartate transaminase (U/l)	40.4±34.0	58.5±64.7	0.213
ALT/Alanine transaminase (U/l)	64.8±118.3	99.3±163.4	0.078

Albumin (g/dl)	3.8±0.5	3.6±0.5	0.048
D-dimer (mg/l)	2.1±8.3	2.8±5.4	0.662
Fibrinogen (mg/dl)	589.8±223.5	590.7±253.4	0.938
Blood glucose (mg/dl)	112.1±48.8	135.6±87.4	0.061
IL-6 (pg/ml)	20.5±4.4	57.1±13.5	0.090
At 12-month follow-up			
Hemoglobin (g/dl)	15.0±1.5	14.2±2.0	0.012
Leukocytes (count x10^3/µl)	6.6±1.8	7.0±1.5	0.633
Neutrophils (count x10^3/µl)	4.0±2.6	4.3±2.9	0.995
Lymphocytes (count x10^3/µl)	2.2±0.9	2.2±0.8	0.536
Ferritin (ng/ml)	173.3±364.5	91.8±76.8	0.023
Platelets (count/µl)	221,820±61,508	256,583±113,230	0.028
C-Reactive Protein (mg/l)	3.5±4.6	4.6±5.6	0.358
Lactate dehydrogenase (U/l)	179.73±34.6	200.3±60.0	0.017
AST/Aspartate transaminase (U/l)	25.8±15.6	22.4±7.5	0.261
ALT/Alanine transaminase (U/l)	28.6±28.0	22.3±13.0	0.134
Albumin (g/dl)	4.7±4.3	4.2±0.3	0.442
D-dimer (mg/l)	0.5±0.6	0.8±1.9	0.217
Fibrinogen (mg/dl)	371.4±435.2	380.9±181.3	0.856
Blood glucose (mg/dl)	97.1±20.5	106.6±41.4	0.091
IL-6 (pg/ml)	6.4±12.8	4.9±10.3	0.413
Vitamin D (ng/ml)	22.7±8.7	19.5±9.2	0.042
Phosphorus (mg/dl)	3.3±1.1	3.4±1.1	0.790
Calcium (mg/dl)	9.5±1.1	9.3±1.0	0.472
Alpha-1 antitrypsin (mg/dl)	124.3±15.4	129.1±18.2	0.480
Alpha antitrypsin range abnormal	1 (1.1)	1 (1.1%)	0.166
Serology, IgG Ab +ve	98 (95.1)	43 (91.5)	0.536

SD: standard deviation.

Tabla S3. Number and proportion of hospitalized patients with normal or impaired lung function at 12 months with plasma determination of alpha-1 antitrypsin (AAT), allelic genotyping (Pi*MM, Pi*MS, Pi*MZ and Pi*MP Lowell) and C-reactive protein (CRP) >5 mg/dl.

Genotype	Lung function at 12 months						p-value
	Normal (n=108)			Impaired (n=49)			
	N (%)	AAT mean ± SD	CRP >5 N (%)	N (%)	AAT mean ± SD	CRP >5 N (%)	
Pi*MM	91 (84.2)	126.4±14.7	15 (20)	40 (81.6)	133.9±14.4	9 (18.4)	0.016
Pi*MS	16 (14.8)	116.3±15.4	5 (4.6)	7 (14.2)	116.7±15.4	0 (0)	0.962
Pi*MZ	0	-	0	2	89.0±21.2	0 (0)	-
Pi*MP Lowell	1 (0.9)	95.0	0	0	-	0	-

Table S4. Results of multiple comparison tests between different alpha-1 antitrypsin (AAT) genotyping variants and respiratory function parameters at 6 months.

Parameter	Genotype		Mean difference	SD	p-value
FEV1 (%)	Pi*MM	Pi*MS	8.4	4.4	0.176
		Pi*MZ	21.6	12.3	0.319
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	13.2	13.8	1.000
FVC	Pi*MM	Pi*MS	-33.1	16.4	0.139
		Pi*MZ	14.7	49.7	1.000
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	47.8	51.6	1.000
FEV1/FVC	Pi*MM	Pi*MS	0.97	2.1	1.000
		Pi*MZ	8.2	6.0	0.508
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	7.3	6.2	0.728
TLC %	Pi*MM	Pi*MS	0.93	3.5	1.000
		Pi*MZ	28.7	10.6	0.023
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	27.8	11.0	0.039
DLCO %	Pi*MM	Pi*MS	4.3	5.2	1.000
		Pi*MZ	41.9	15.5	0.024
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	37.5	16.1	0.064
KCO %	Pi*MM	Pi*MS	-0.21	4.3	1.000
		Pi*MZ	20.5	12.8	0.335
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	20.7	13.3	0.364
RV %	Pi*MM	Pi*MS	-13.8	6.4	0.099
		Pi*MZ	30.9	18.9	0.316
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	44.7	19.7	0.075
6MWT Distance (m)	Pi*MM	Pi*MS	21.7	24.9	1.000
		Pi*MZ	210.2	74.9	0.017
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	188.5	77.7	0.050
6MWT Initial SaO2 (%)	Pi*MM	Pi*MS	0.02	0.38	1.000
		Pi*MZ	1.6	1.14	0.492
		Pi*MP Lowell	-	-	

	Pi*MS	Pi*MZ	1.6	1.2	0.558
6MWT Final SaO2 (%)	Pi*MM	Pi*MS	-0.01	0.88	1.000
		Pi*MZ	5.5	2.6	0.140
		Pi*MP Lowell	-	-	-
	Pi*MS	Pi*MZ	5.5	2.7	0.118

FVC=forced vital capacity; FEV1=forced expiratory volume in the first second; TLC=total lung capacity; RV=residual volume; DLCO=diffusing capacity for carbon monoxide; 6MWT=six minute walk test; SaO2=oxygen saturation; SD= standard deviation.

8. DISCUSIÓN:

8.1. Artículo 1: Asociaciones únicas y múltiples de las secuelas post-agudas del COVID-19: Estudio de cohorte prospectivo de 6 meses de duración.

Este estudio hace un análisis exhaustito del desarrollo de condición PCC 6 meses después del proceso agudo y, de la influencia que pueden tener en el desarrollo de esta (ya sea por sintomatología persistente y/o presencia de secuelas orgánicas variables) las variables de género, la gravedad de la enfermedad durante la fase aguda y, la presencia de comorbilidades respiratorias previas.

Respecto a la clínica persistente, hasta un 73,3% de nuestros pacientes a los 6 meses seguían presentando algún síntoma o afirmaban haber desarrollado nueva clínica tras el proceso agudo no atribuible a diagnósticos alternativos. A los 6 meses, dentro de los variables descriptivos de la clínica y la salud mental; la disnea, el cansancio, la tos seca, la anosmia, la ageusia, la cefalea, la alopecia, las alteraciones cognitivas y, la afectación emocional con desarrollo de depresión secundaria eran las características más prevalentes persistentes. Respecto a los índices de laboratorio, las alteraciones en los parámetros de la hemoglobina, el porcentaje de linfocitos, de neutrófilos, plaquetas, bilirrubina total, creatinina, proteínas totales, ALT, glucosa, LDH, ferritina y DD se seguían objetivando. Esto concuerda con los hallazgos de estudios previos a largo plazo del SARS[107-109] y el MERS [110-111]. Además, hemos demostrado que hay asociación entre PCC y el sexo del paciente. En particular, el sexo femenino es favorable para la persistencia de sintomatología, coincidiendo con hallazgos previos[112].

Para valorar las secuelas orgánicas pulmonares post COVID-19 en el seguimiento se realizaron pruebas funcionales respiratorias y TCAR de tórax. Encontramos que las PFR se vieron afectadas en pacientes post COVID-19 independientemente de si tenían comorbilidades respiratorias previas o no. Se objetivaron alteraciones funcionales con patrones restrictivos, obstructivos y capacidad de difusión alterada. El parámetro más frecuente afectado la DLCO. Su disminución puede sugerir una enfermedad pulmonar intersticial difusa (EPID) incipiente o la presencia de anomalías vasculares pulmonares secundarias a la COVID-19[113-114]. Dichos hallazgos son consistentes con las imágenes de TCAR que mostraban opacidades en vidrio deslustrado, con afectación reticular subpleural y bronquiectasias por tracción. Sin embargo, en el TCAR no había imágenes de bronquiolitis descritas. Estos resultados están en consonancia con lo publicado hasta ese momento del SARS-CoV-2[115-116] y con lo descrito por otros coronavirus previos. En concreto, en el SARS-CoV describían un deterioro de la función pulmonar del 25-50% entre 6

meses y 3 años después del alta[117]. En el caso del MERS objetivaron secuelas funcionales en hasta el 37% de los supervivientes a los 12 meses con deterioro de la DLCO[118].

En el análisis de la presencia de comorbilidad respiratoria previa (ERP) frente a la PCC, encontramos que los pacientes con ERP deben ser monitorizados a largo plazo, sobretodo aquellos con IMC anormal, exfumadores y con disnea basal pues están más predispuestos a desarrollar dicha condición.

En el análisis del estado de hospitalización frente al desarrollo de PCC, se demuestra que el haber pasado la infección de forma leve (sin necesidad por tanto de hospitalización), o de forma moderada/grave (con necesidad de hospitalización) es independiente para el desarrollo de la PCC. En el análisis bivariado se obtuvieron las siguientes características con asociación robusta y relaciones significativas entre hospitalización y PCC: sexo masculino, mayor de 59 años, no trabajador en el área sanitaria y eventos tromboembólicos venosos. No obstante, tras el análisis multivariante, aumentó la robustez de la asociación para los predictores, excepto para la edad > 59 años, añadiendo la fatiga como nueva característica significativa. La influyente asociación negativa de eventos tromboembólicos, y la fatiga sugiere que los pacientes sin hospitalización también necesitan un seguimiento a largo plazo.

Por todo esto, en el seguimiento post COVID podemos distinguir dos grupos de pacientes. El primero, compuesto mayoritariamente por hombres, mayores de 59 años, con IMC cercano al sobrepeso, que han requerido hospitalización por neumonía covid-19 y presentan síntomas persistentes con lesiones pulmonares objetivas, ya sean radiológicas o en pruebas funcionales. Y, el segundo grupo, formado mayoritariamente por mujeres, menores de 55 años, con peso normal, que no han requerido ingreso hospitalario en su mayoría y, por pasar la infección de forma leve (sin neumonía) en el momento del proceso agudo, pero que consultan por la aparición de síntomas novo o persistencia de clínica ya existente y, que tienen muy ligera afectación funcional residual. No obstante, no debemos menospreciar las alteraciones funcionales respiratorias en este grupo, pues incluso con imágenes de tórax normales, la DLCO puede estar disminuida indicando alteraciones de la membrana alveolocapilar.

Fortalezas del estudio: La principal fortaleza de nuestro estudio es el seguimiento a medio plazo de los pacientes post covid-19, incluyendo pacientes con diferente gravedad de la enfermedad, a diferencia de la mayoría de los manuscritos publicados hasta esa fecha donde solo consideran pacientes hospitalizados para estudios de cohortes, lo que puede inducir un sesgo importante. Además, este estudio es el primero en presentar caracterizaciones únicas y múltiples de las

secuelas a largo plazo de COVID-19, siendo por tanto, uno de los más detallados y prolongados publicados.

Limitaciones del estudio: La mayor disposición de los pacientes sintomáticos a participar en un estudio de seguimiento es un posible factor de sesgo, como en todos los estudios observacionales. Los hallazgos del estudio pueden estar limitados por ser unicentro. Entendemos esto como posible sesgo de localización.

8.2. Artículo 2: PET/CT con [18F]FDG en las complicaciones a corto plazo de COVID-19: Marcadores metabólicos de inflamación persistente y deterioro de la función respiratoria**.**

En este estudio se analizó la utilidad del [^{18}F]FDG-PET/TC para detectar procesos inflamatorios residuales después del COVID-19, contribuyendo así al conocimiento de los procesos fisiopatológicos que subyacen a la condición post COVID.

Históricamente, la investigación sobre el papel de [^{18} F]FDG-PET/CT en la infección por COVID-19 se ha centrado generalmente en la fase aguda. En este sentido, Qin et al [119]. informaron de una alta captación de [^{18} F]FDG en lesiones pulmonares y ganglios linfáticos mediastínicos en pacientes con sospecha de infección COVID-19, y Colandrea et al[120] describieron una serie de pacientes oncológicos asintomáticos pero diagnosticados de COVID-19 con un aumento de la captación de [^{18} F]FDG en lesiones pulmonares. Sin embargo, pocos estudios han abordado el papel del PET TC en el seguimiento post COVID; Dietz et al[121] informaron de la captación de [^{18} F]FDG en lesiones pulmonares y ganglios linfáticos mediastínicos de pacientes con COVID-19 en los días 6 a 14 después del inicio de los síntomas. Johnson et al [122] propusieron que la captación alta de [^{18} F]FDG en los ganglios linfáticos mediastínicos podría ser secundaria a la afectación pulmonar en COVID-19. Y, otros estudios informaron de aumento de la actividad metabólica en lesiones pulmonares residuales en sobrevivientes de COVID-19[123-124]. Nuestros resultados están en línea con los hallazgos anteriores y aportan nuevos datos sobre el aumento de la actividad metabólica en el tejido pulmonar de aspecto normal y en los ganglios linfáticos mediastínicos de tamaño normal. La captación elevada de [^{18} F]FDG reflejaría una mayor actividad glucolítica por infiltración e inflamación del pulmón, incluso en áreas normalmente aireadas que no presentan alteraciones morfológicas en las imágenes de TC, demostrando la mayor capacidad de [^{18} F]FDG-PET/TC para detectar áreas pulmonares inflamadas en comparación con la TC sola[125], que pueden persistir tiempo después de la desaparición de la enfermedad COVID-19. La posible duración de la respuesta inflamatoria posterior a la COVID-19

en los pulmones y los sitios extrapulmonares aún no se ha establecido y justifica una mayor investigación.

A los 2-3 meses del alta, los pacientes que continuaban con captación elevada de [^{18} F]FDG en el tórax eran; los de edad avanzada e índice de Charlson elevado; aquellos con mayor cansancio y dificultad respiratoria al ingreso y; recuentos más bajos de hemoglobina y linfocitos. El SUV pico de la lesión diana y el TLG pulmonar se correlacionaron significativamente con los reactivos de fase aguda y los recuentos de glóbulos blancos al ingreso, durante la estancia hospitalaria y 2-3 meses después del alta. Aunque faltan estudios similares en sobrevivientes de COVID-19 gravemente enfermos para comparar estos resultados, son consistentes con hallazgos previos sobre factores de riesgo para infecciones más graves, incluida la edad avanzada, comorbilidades subyacentes[126-127], y cambios similares en los recuentos de glóbulos blancos, recuentos de linfocitos, niveles de procalcitonina y CRP, y NLR [127-128]. La persistencia a lo largo del tiempo de una mayor intensidad de captación de [^{18} F]FDG puede reflejar una fase aguda más grave de la enfermedad.

La mayoría de los pacientes del estudio no mostraron lesiones pulmonares en las tomografías computarizadas 2 a 3 meses después del alta; sin embargo, la función pulmonar se vio afectada en más de la mitad de los pacientes con un TC de pulmón normal. La proporción de nuestros pacientes con deterioro de la función pulmonar a los 2 o 3 meses estuvo en línea con lo descrito en la literatura. La variable más afectada fue la DLCO, como se informó en estudios previos, lo que puede indicar la presencia de afectación intersticial [125,131-133].Por lo tanto, las pruebas de función pulmonar y [^{18} F]FDG-PET/CT son más sensibles que la TC sola para identificar candidatos para la rehabilitación pulmonar después del COVID-19[134].

Una captación alta de [^{18} F]FDG puede estar relacionada con un aumento de la glucólisis anaeróbica causada por una cascada de reacciones que involucran a las células inflamatorias [67,135]. De esta forma, la captación de [^{18} F]FDG por lesiones pulmonares y ganglios linfáticos observada en este estudio puede deberse a una activación inmunitaria o inflamatoria inespecífica, similar a la elevada captación de [^{18} F]FDG observada en lesiones pulmonares causadas por MERS, virus de la influenza pandémica H1N1 y neumonía organizada[136-138].

Fortalezas: Primer estudio hasta la fecha de publicación que analiza el papel del PET TC (prueba nuclear que aporta información morfológica y funcional) en el seguimiento de los pacientes post-COVID a medio plazo, intentando aportar información fisiopatológica sobre la PCC.

Limitaciones: La principal limitación de este estudio fue la ausencia de un grupo de control, además del tamaño muestral reducido. Evidentemente, el uso de PET/TC en pacientes sanos

colaboradores no estaba autorizada. Además, no se disponía de resultados de las pruebas de función respiratoria basal de los pacientes antes de la COVID-19, aunque la presencia de enfermedad pulmonar crónica era un criterio de exclusión. Otra limitación del estudio fue la ausencia de un período de seguimiento para explorar la relevancia clínica a largo plazo del deterioro de la función respiratoria.

8.3. Artículo 3: Niveles anormales de Alfa-1 antitripsina y otros factores de riesgo asociados con el deterioro de la función pulmonar a los 6 y 12 meses tras la hospitalización por COVID-19: estudio de cohortes.

Este estudio evidencia cuales son los factores de riesgo que pueden predisponer al deterioro de la función pulmonar un año después de ser hospitalizados por COVID-19.

Las variables asociadas de forma estadísticamente significativa con el deterioro funcional a los 12 meses de la hospitalización eran; la edad avanzada, alto índice de Charlson y presencia de ≥ 2 comorbilidades. La presencia de DM-2, la neumopatía basal y el EPOC eran los antecedentes personales que alcanzaban también dicha significación. La presencia de disnea y/o astenia, neumonía en la radiografía de tórax con distribución mixta y/o central, desarrollo de SDRA, ingreso en UCI, necesidad de IOT, mayor estancia hospitalaria, tratamiento en bolos de metilprednisolona y/o tocilizumab, junto con la elevación de parámetros inflamatorios en el ingreso de recuento de leucocitos, neutrófilos, plaquetas, índice NLR, y LDH también mostraron dicha asociación significativa. Estos datos están en consonancia con lo publicado hasta ahora de factores de riesgo asociados al desarrollo de peor curso clínico, SDRA y muerte durante la hospitalización[139].Tras el ajuste del modelo multivariante las variables predictoras independientes que explicarían las diferencias observadas entre ambos grupos son el índice de Charlson elevado, la radiografía de tórax con presencia de neumonía de distribución central y/o mixta, la anemia en el ingreso, la estancia en UCI, la necesidad de tratamiento corticoideo en bolos intravenosos, la persistencia de auscultación respiratoria anormal en la revisión de los 6 meses y la elevación de LDH en la revisión de los 12 meses.

También se objetivó cómo 49 pacientes (31.2%) mantenían un deterioro de la función pulmonar a los 12 meses, siendo los parámetros funcionales más afectados el FEV1 y la DLCO. Esto es similar con lo publicado por otros estudios sobre secuelas funcionales a corto-medio plazo hasta ese momento[140-142] y, con lo publicado a largo plazo secundario al SARS y MERS, donde se describen hasta 5 años después de la infección aguda deterioro de la capacidad funcional. En general, en nuestro trabajo todas las variables funcionales mejoraban de los 6 a los 12 meses,

pero sólo alcanzan la significación estadística la TLC y, la distancia recorrida en metros en el TM6M que muestra una disminución estadísticamente significativa, probablemente por persistencia de debilidad muscular a pesar de la mejora de los volúmenes pulmonares, lo cual puede provocar disnea de esfuerzo con limitación del rendimiento en dicha prueba.

Respecto a la AAT, observamos que 2 pacientes tenían rango anormal de la misma (ambos con niveles inferiores a la normalidad), uno perteneciente al grupo de recuperados y otro al grupo de no recuperados funcionalmente. Los niveles medios de dicha proteína en los recuperados eran de 124,26±15,52 y en los no recuperados de 129,07±18,20 sin diferencias significativas. No obstante, había 28 pacientes con niveles normales de AAT y PCR >5mg/L. Recordamos que la proteína AAT es un reactante de fase aguda y se ha demostrado que sus niveles plasmáticos pueden estar incrementados 2-3 veces en respuesta a estímulos inflamatorios o infecciosos, de manera similar a la PCR [143].Por ello, a estos pacientes se les solicito la secuenciación genómica completa sin encontrar patogenicidad en la variante encontrada.

Las variantes alélicas de la AAT de nuestros pacientes se distribuyen de forma similar a la población general[144]. Cabe destacar que los dos casos existentes de PiMZ y, la mitad de los casos de PiMS estaban en el grupo de pacientes con deterioro funcional. En el análisis de comparaciones múltiples al confrontar el fenotipo PiMM frente al PiMZ, vemos que los pacientes con PiMZ tenían niveles inferiores de TLC, DLCO y distancia recorrida en el TM6M alcanzando la significación estadística.

Fortalezas: Importante tamaño muestral. Analiza la relación entre el deterioro funcional pulmonar a largo plazo y ciertas características como el DAAT y sus alteraciones genéticas aún pendientes de discernir papel en el COVID-19 y las secuelas de la misma.

Limitaciones: Al ser un estudio unicentro y observacional puede existir un sesgo de localización.

9. CONCLUSIONES:

PRIMERA**:** Tras 6 meses de la infección aguda por SARS-CoV-2, se verifica la presencia de condición post COVID-19 entre nuestros pacientes debido o bien a la persistencia de síntomas desde el proceso agudo y/o aparición de otra clínica de novo o a las secuelas orgánicas pulmonares.

SEGUNDA: El desarrollo de condición post COVID-19 es independiente de la gravedad de la enfermedad durante la fase aguda y, el tener o no comorbilidades respiratorias previas. No obstante, sí esta influenciado por el género. En concreto, se objetiva como el sexo femenino es un factor favorable para la persistencia de la sintomatología.

TERCERA: Las alteraciones pulmonares radiológicas se mantienen hasta en un tercio de los pacientes al mes de seguimiento, siendo el vidrio deslustrado y el patrón reticular los hallazgos más frecuentes en la TACAR de tórax.

CUARTA: Las alteraciones funcionales respiratorias se observan en pacientes con y sin enfermedad pulmonar previa y, reflejan la importancia de la difusión pulmonar como el parámetro funcional más frecuentemente afectada en el seguimiento a medio plazo.

QUINTA: El PET/TAC con 18-fluoro-dexosi-D-glucosa ([^{18} F]FDG) ofrece un enfoque complementario a otras modalidades de imagen en el seguimiento post COVID al proporcionar información morfológica y funcional mediante la actividad metabólica.

SEXTA: La captación elevada de [^{18} F]FDG a corto plazo reflejaría una mayor actividad glucolítica por infiltración e inflamación del pulmón, incluso en áreas normalmente aireadas que no presentan alteraciones morfológicas en las imágenes de TAC, demostrando su mayor capacidad para detectar áreas pulmonares inflamadas en comparación con la TAC sola.

SÉPTIMA: Las pruebas de función respiratoria junto con el PET-TAC con [^{18}F]FDG son más sensibles que la TAC sola para identificar candidatos con secuelas pulmonares después del COVID-19.

OCTAVA: El valor de captación estandarizada máximo de la lesión diana y el de glucolisis tumoral total pulmonar se correlacionaron significativamente con los reactivos de fase aguda y los recuentos de glóbulos blancos al ingreso, durante la estancia hospitalaria y 2-3 meses después del alta.

NOVENA: Hasta un tercio de los pacientes que sufren COVID-19 grave mantienen un deterioro de las pruebas funcionales respiratorias 12 meses tras la hospitalización.

DECIMA: Entre las características basales que pueden predisponer al deterioro funcional respiratorio se encuentran: las cifras bajas de alfa 1 antitripsina o variantes alélicas deficientes, en concreto la presencia de Pi*MZ; el tener un índice de Charlson elevado; la presencia al ingreso hospitalario de neumonía grave con anemia, la necesidad de bolos de corticoides y/o el ingreso en UCI.

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11. ANEXOS:

11.1 Artículo 1.

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On the single and multiple associations of COVID-19 post-acute sequelae: 6-month prospective cohort study

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Medical research is progressing to clarify the full spectrum of sub-acute and long-term effects of the post-COVID-19 syndrome. However, most manuscripts published to date only analyze the effects of post-COVID-19 in patients discharged from hospital, which may induce significant bias. Here, we propose a pioneering study to analyze the single and multiple associations between post-COVID-19 characteristics with up to 6-months of follow-up in hospitalized and non-hospitalized COVID-19 patients. The cohort study was conducted from May to October 2020 at the University Hospital Virgen de la Nieves, the leading hospital assigned for patients with COVID-19 in Granada, Spain. A total of 372 and 217 patients—with 217 and 207 included in the first and second follow-up visits—were referred 2 and 6 months after diagnosing COVID-19, respectively. We find out that post-COVID-19 clinical and mental health impairment symptoms are correlated with patient gender. Logistic adjustments showed strong statistically robust single and multiple associations of demographic, clinical, mental health, X-ray, laboratory indices, and pulmonary function variables. The functional lung tests are good predictors of chest CT imaging abnormalities in elderly patients. Bilateral lung involvement, subpleural reticulum, ground-glass opacity, peripheral lung lesions, and bronchiectasis were the most common findings of the high-resolution computed tomography images. Non-hospitalized patients suffer more severe thromboembolic events and fatigue than those hospitalized.

Abbreviations

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SARS-1	Severe acute respiratory syndrome
MERS	Middle East Respiratory Syndrome
PCS	Post-COVID-19 syndrome
ICAT	Coalition Against Tobacco
BMI	Body mass index
FFuC	First follow-up consultation
SFuC	Second follow-up consultation
X-Ray	Chest radiograph
CT	Chest computed tomography
HRCT	High-resolution computed tomography

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GGO	Ground glass opacity
DILD	Diffuse interstitial lung disease
PFT	Pulmonary function test
FEV1	Forced expiratory volume in the first second
FVC	Forced vital capacity
FEV1/FVC	The FEV1/FVC ratio
RV	Residual volume
FRC	Residual functional capacity
TLC	Total lung capacity
DLCO	Carbon monoxide transfer by a single breath
KCO	Diffusion constant for carbon monoxide
6MWT	6-Minute walking test
SERAM	Spanish Society of Medical Radiology
IQR	Interquartile range
CIs	Confidence intervals
ORs	Odds ratios
BVA	Bivariate analysis
MVA	Multivariate analysis
PRD	Pre-existing respiratory disease
RT-PCR	Reverse transcription-polymerase chain reaction
PE	Pulmonary embolism

Long-term effects on multiple organ systems, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—pathogen of coronavirus disease 2019 (COVID-19)—is one of the current problems faced by patients after passing the disease[1–3]. Preliminary studies report persisting symptoms of SARS-CoV-2, such as fatigue, dyspnea, cognitive deficit, arthralgia, impaired lung functions, and abnormal chest images[4–10]. Similar persistent symptoms were reported in patients from previous coronavirus nfections—including the 2003 SARS epidemic and the 2012 Middle East Respiratory Syndrome (MERS)[11–16]—reinforcing concerns about post-COVID-19 syndrome (PCS)[17] (see definition of PCS in materials and methods). The work of Ongsobre et al. clearly shows the persistent and prolonged effects of lung function impairment one year after acquiring severe acute respiratory syndrome (SARS1)[15]. In addition, Liu et al. point out that deterioration of pulmonary functions and quality of life may occur up to 3 years after acute infection[18].

Due to the limited capacity of the hospitals, only a tiny fraction of people with COVID-19 are admitted to hospitals[19–22]. However, most manuscripts published—to date—only consider hospitalized patients for cohort studies, which may induce significant bias[23]. Current 12-, 6- and 3-month follow-up studies focus on persistent clinical, psychological, pulmonary function, physical problems, and chest CT imaging only for the discharged patients[8,10,24–29]. No study has yet reported the scope of post-acute sequelae of COVID-19 in a singular or multiple manners, including the non-hospitalized patients. Also, the association between pre-existing respiratory diseases and PCS is still unknown.

Our goal is to analyze the degree of single and multiple associations between clinical characteristics, demographic features, mental health, and pulmonary function test linked to PCS—of the first variant of SARS-CoV-2—in patients with/without previous respiratory diseases, hospitalized or not, and the abnormalities of chest CT images.

Results

Of the 217 patients—of which 116 (53.5%) were male—with SARS-CoV-2 was examined, including hospitalized and not hospitalized patients. These patients were monitored from May to October 2020. The follow-up study from May to October 2020 was divided into two follow-up consultations. The FFuC was carried out two months after the diagnosis of infection—from May to mid-July 2020—and the SFuC six months after the initial diagnosis—from July to October 2020. A total of 148 patients were excluded from the study for the reasons set out in Fig. 1. The median and interquartile range for age and BMI were 59 (49–68) and 28 (26–32), respectively. Active smokers or ex-smokers 89 (41%) with International Coalition Against Tobacco (ICAT) of 0[1–2]. 52.6% had been in contact with family members with suspected or confirmed COVID-19. At the FFuC, the most prevalent symptoms were dyspnea in 138 (53.6%) together with fatigue116 (53.5%), emotional affectation 117 (53.9%) and depression 124 (57.1%). In 64 patients (30.3%), the abnormal radiological findings continued. These and those with stress dyspnea were asked for chest HRCT. In the SFuC, 154 patients (73.3%) still showed symptoms or claimed to develop new symptomatology after the acute process that was not attributable to alternative diagnoses. Dyspnea 88 (42.5%), fatigue 99 (47.8%), hair loss 47 (22.7%), emotional affectation 91 (44%), and depression 45 (21.7%) were the most frequent symptoms. However, other alterations such as memory, concentration, and language deficits started to appear after the FFuC, reflecting a global cognitive deficit of up to 56 (27.1%). They expressed it as a lack of mental fluency with stuttering and "brain fog"[30]. Also, erectile dysfunction or decreased sexual appetite was present in 3 (1.4%)—not plotted. The overall results of the FFuC and SFuC are shown in Figure S1 of the supplementary material.

Clinical outcomes and laboratory indices in female and males. Figure 2 shows the clinical features, physical examination, and mental health of females/males with PCS—red affected and blue healthy—for first/second follow-up consultations. At the FFuC, the most frequent symptom—over 25%—in females [males] were dyspnea in 69 (68.3%) [69 (40.5%)], fatigue 61 (60.4%) [55 (47.4%)], emotional affectation 60 (59.4%)

Figure 1. Timeline and flowchart illustrating the series of events in chronological order with the actions and tests conducted after the first case detected at the University Hospital Virgen de las Nieves. Starting with the first COVID-19 test results, the number of patients referred to post-COVID-19 consultations, the details of the first and second consultations, and data analysis.

[57 (49.1%)], and depression 36 (35.6%) [88 (75.9%)], see Fig. 2A. Note here that in females, the symptoms of dyspnea, fatigue, and emotional affectation have a greater influence than in males, except for depression which affects more males than females. There was a gender difference for symptoms of arthralgia, fever, and hair loss. The rest of the less frequent symptoms—except epiphora—were still present after six months of the acute process. Figure 2B shows the results for the SFuC where the significant features for females [males] were dyspnea 46 (48.4%) [42 (37.5%)], fatigue 58 (61.1%) [41 (36.6%)], hair loss 42 (44.2%), emotional affectation 52 (54.7%) [39 (34.8%)], and depression 34 (35.8%). All these symptoms are more frequent in females than in males. From the p-values, we conclude that whether a person presents a lack of energy, emotional affectation, depression, or cognitive deficit depends on the person's gender. Also, decreased sexual appetite was observed in 1 female patient and erectile dysfunction in 2 males—not plotted.

The normal (N, green color) and abnormal (Abn., yellow color) laboratory indices—antibodies, hematologic, biochemical, infection and coagulation—are shown in Fig. 3 for females and males. At the FFuC—Fig. 3A, a positive IgG and IgM were presented in 77 (80.2%) [95 (90.5%)] females [males] patients. This result confirms that both women and men have passed a relatively recent infection and are developing antibodies—as expected. A negative IgG and positive IgM or a positive IgG and negative IgM were presented in less than 25% of females and males. Also, Fig. 3A,C show this cohort's most relevant abnormal indices in females [male] patients. Hemoglobin 34 (31.8%) with 16 (15.7–16.4)—men only, creatine 26 (30.2%) with a median of 0.62 (0.57–0.64), ferritin 25 (32.1%) [73 (70.9%)] with 160 (132–213) [259 (173–405)] and D-dimer 26 (38.2%) [36 (38.3%)] with 0.97 (0.66–1.9) [0.83 (0.68–1.6)]. The hemoglobin and serum ferritin indices reject the chi-square null hypothesis of independence regarding gender. The antibody test of the SFuC—Fig. 3B—shows that a positive IgG and IgM were presented in 44 (48.9%) [47 (45.6%)] females [males] patients. Also, 40 (44.4%) [51 (49.5%)] of females [males] were positive with IgG and negative IgM, e.i., the patients have been in contact with the virus and have generated antibodies after six months. Here all the frequency of abnormal laboratory indices for females is less than 25%. However, more than one-quarter of male patients had abnormal hemoglobin [49 (47.6%) with 16.2 (15.7–16.6)] and more than half abnormal ferritin [57 (57%) with 198 (163–328)]. The hemoglobin, serum ferritin indices—similar to the FFuC, and the total bilirubin reject the chi-square null hypothesis of independence regarding gender. The median and the interquartile range for all the laboratory indices for females and males are represented in Fig. 3C. Here, the abnormal values of the indices for the different genera are clearly shown.

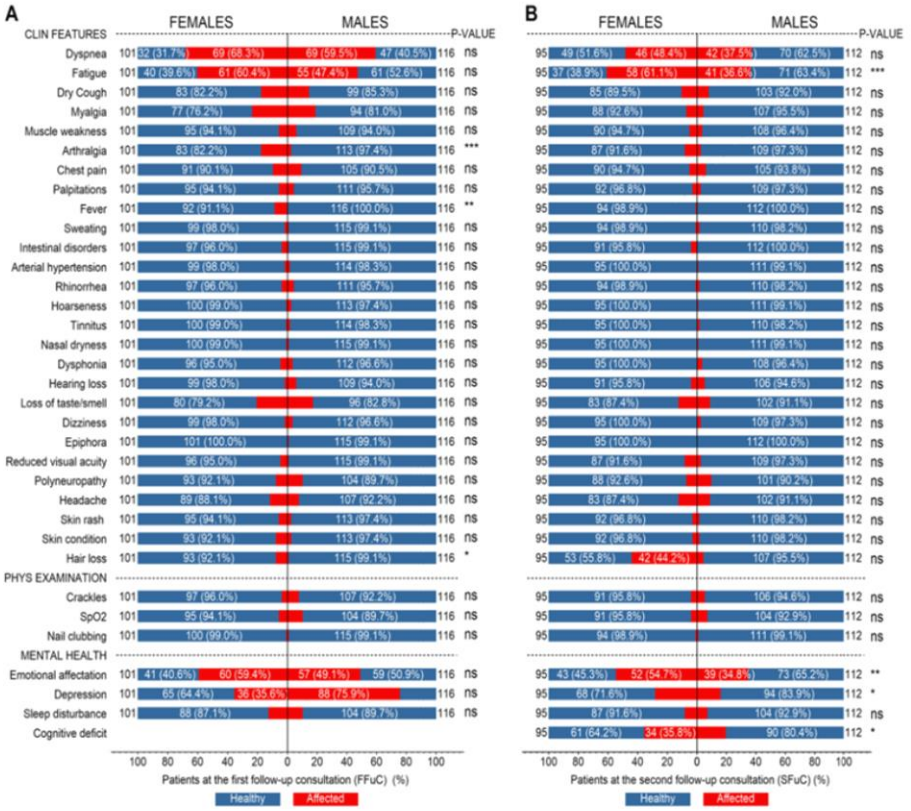

Figure 2. Clinical features, physical examination, and mental health of females and males with post-acute sequelae of SARS-CoV-2 for the first and second follow-up consultations—2- and 6-months post symptom onset of COVID-19. Frequencies of symptoms presented in N° (%) of the total for each gender in the FFuC (**A**) and SFuC (**B**).

Association between PCS features with PRD and hospitalization. Figure 4 shows the OR of PCS at 6-month follow-up using BVA and MVA, given the presence of a pre-existing respiratory disease (PRD) and hospitalization. A total of 207 patients were used for the BVA of gender, demographic characteristics, clinical features, mental health diseases, and hospitalization, of which 46 had the PRD. Also, we applied a BVA for lung exploration tests in a total of 157 patients, of which 33 had a PRD. For the MVA, we used 155 patients for all predictors, of which 33 had a pre-existing respiratory disease (see Fig. 4A). In addition, two hundred seven patients were used for the BVA with the same variables—except hospitalized—used for PRD analysis, of which 173 had been hospitalized. Also, for the BVA of the lung examination tests, 130 patients—of 157—had been admitted to the hospital. The MVA included 33 patients—of 155—that were discharged from the hospital (see Fig. 4B). The descriptive analysis, medians, and IQR of all the variables are shown in Figures S2 and S3 of the Supplementary Information.

After the bivariate adjustment, the following variables are in the robust (i.e., $2 \leq OR$) range of ORs positively associated with a PRD compared to those without PRD: abnormal BMI, dyspnea, fatigue, emotional affectation, depression, impairment of FVC, FEV1, VR, DLCO, and FEV1/FVC—below their normal limits. The remaining features are in the range of less impressive OR values (i.e., $0.5 < OR < 2$). Note that the highest OR corresponds to the FEV1/FVC ratio, implying that it has the highest bivalent association with the PRD compared to without PRD. Only the ORs of the abnormal BMI, dyspnea, fatigue, emotional affectation, depression, FEV < 80%, DLCO < 80%, and FEV1/FVC < 70% ratio are statistically significant (p values < 0.05). The ORs for abnormal BMI, ex-smokers, non-health workers, dyspnea, dry cough, loss of taste/smell, headache, emotional affectation, depression, all the impaired lung exploration tests, and the positive hospitalized status are in the positive or negative (i.e., $2 \leq OR$ or $OR \leq 0.5$) robust association range. The ORs of the remaining predictors are in a non-robust range. Statistically significant associations were found for abnormal BMI, ex-smokers, and dyspnea, as shown in Fig. 4A.

After the BVA, in the range of robust OR scores positive associated to hospitalized compared to non- hospitalized patients are the following features: male sex, age > 59 years, active-smoker, ex-smokers, non- health worker, the impairment of FVC, FEV1, and 6MWT. Venous thrombosis is the only predictor with a negatively associated

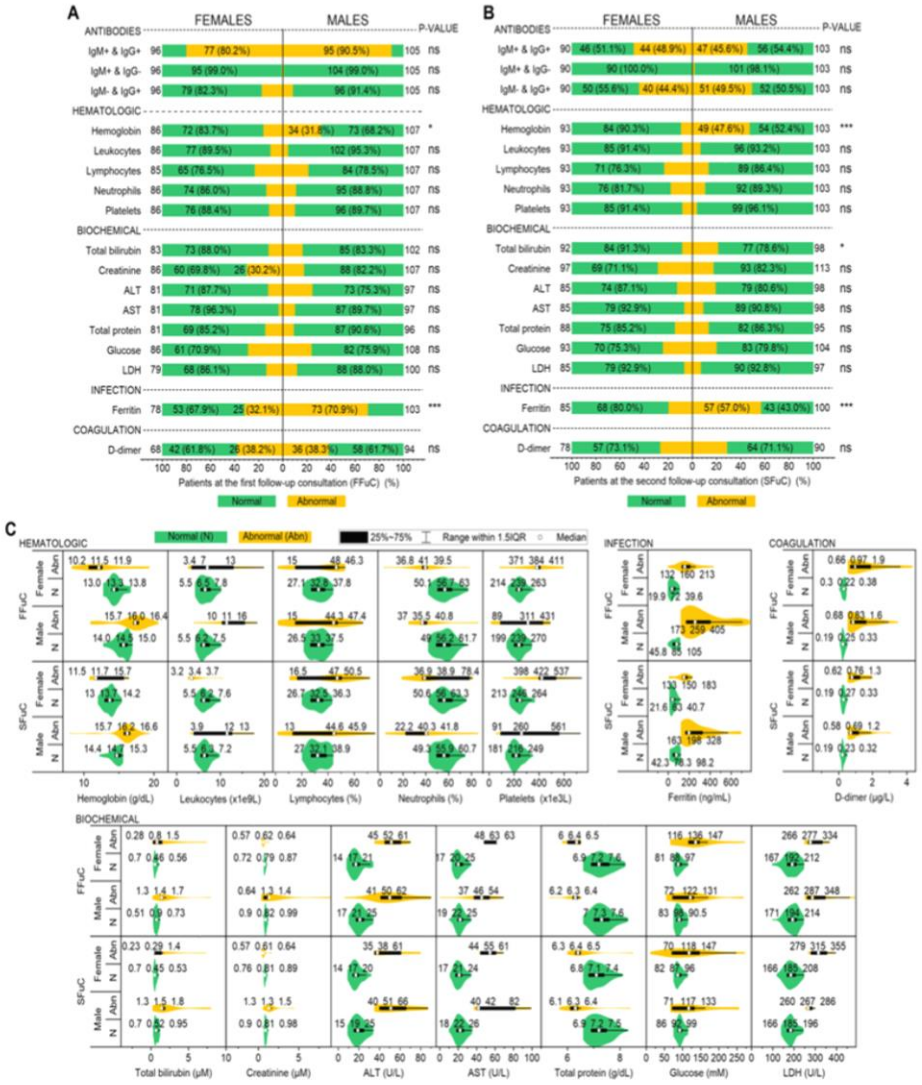

Figure 3. Laboratory indices of females and males with PCS for first/second follow-up consultations. Frequencies of normal (green color) and abnormal (yellow color) laboratory indices presented in N° (%) of the total for each gender for the FFuC (**A**) and SFuC (**B**). (**C**) Violin plots show the laboratory indices' distribution, median, and quartiles for FFuC, SFuC, and gender.

with hospitalized patients. The explanation for this result is that pharmacological thromboprophylaxis was provided during hospitalization. The remaining variables after the BVA are in the less impressive range of ORs, implying a negative association with hospitalized patients compared to non-hospitalized patients. Statistically significant associations were found for gender, age > 59 years, non-health workers, and venous thrombosis (see Fig. 4B). After the MVA, the values of ORs for the male sex, non- health workers, age < 59 years, fatigue, loss of taste/smell, headache, hair loss, venous thrombosis, depression, impairment of FEV1, KCO, and 6MWT are in the range of strongly associated scores to the hospitalization status. The remaining variables are in the interval of non-robust OR values. Predictors of males, non-health workers, fatigue, and venous thrombosis contribute significantly to the MVA (see Fig. 4B).

Association between PCS features with chest CT scan findings. The evolution of CT scans at the same level for a patient—a 65-year-old woman—at 0, 2, and 6 months after COVID-19 is depicted in Fig. 5A–C, respectively. At 0-months (Fig. 5A), the patient had patchy and bilateral ground-glass opacities (single arrows)

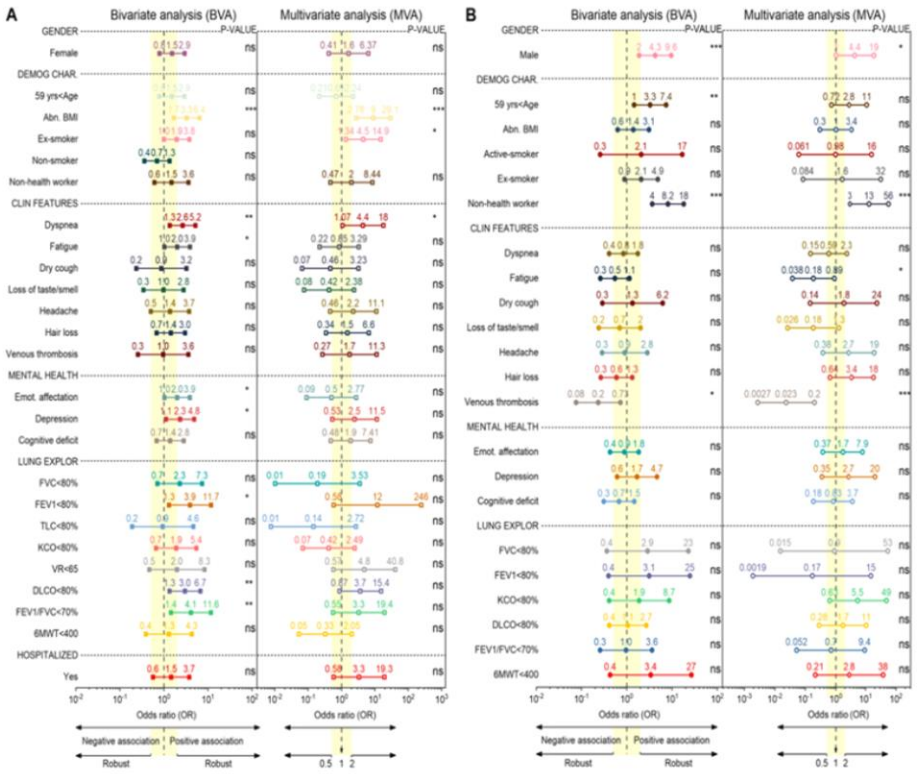

Figure 4. Forest plot of the odds ratio and its CIs (95%) values for the bivariate and multivariate analysis. Relationship between the PCS features with the PRD (**A**) and hospitalization (**B**). The vertical yellow band delimits the regions for a robust (OR ≤ 0.5 or 2 ≤ OR) or weak association (0.5 < OR < 2). The p values of the right column indicate the level of significance.

and intralobular reticular pattern of peripheral subpleural distribution (double arrows). 2-months after COVID-19 (Fig. 5B), lesions have been significantly reduced but still have some residual reticular lesions (double arrows). Figure 5C shows the persistent subpleural banded reticular lesions (double arrow) in posterior segments of inferior lobes after 6-months of follow-up. See Figure S4A–D of the Supplementary Information for additional lung lesions from CT images of 4 patients.

Figure 5D shows the chest high-resolution CT findings of 130 patients, of which 74 (56.9%) presented chest image abnormalities due to consequences of COVID-19. The most notable—over 25%—features were: 68 (52.3%) had bilateral pulmonary involvement, 37 (28.5%) showed a subpleural reticular pattern, 56 (43.1%) had a peripheral distribution, and 59 (45.4%) exhibited a ground-glass opacity pattern. Less frequent CT findings were: unilateral lung involvement and interlobular reticular in 6 (4.6%) patients, central (8 [6.2%]) and central-peripheral (7 [5.4%]) lesion location, mixed GGO (3 [2.3%]), bronchiectasis (21 [16.2%]), emphysema (9 [6.9%]), PE (7 [5.4%]), laminar atelectasis (2 [1.5%]), and halo sign, pleural calcification, residual cavity, intrapulmonary node in 1 [0.8%] patient (see Fig. 5A).

Figure 5B shows the odds ratio after bivariate and multivariate logistic adjustments to measure the association of gender, demographic characteristics, lung examination, pre-existing diseases, and hospitalization status with the abnormal CT outcome. A total of 109 patients—of 130—were used for the BVA and MVA, of which 62 had abnormal CT results. The descriptive analysis, medians, and IQR of all the variables are shown in Figure S4 of the supplementary document. In bivariate analysis, males, age > 59 years, ex-smokers, abnormal radiography, impaired FEV1/FVC ratio, and a positive hospitalized status show a robust positive association (i.e., OR > 2). Non-smokers exhibit a robust negative OR, and the remaining variables are in the range of less impressive OR scores. Male sex, age > 59 years, non-smoker, ex-smoker, and abnormal radiography are statistically significant. Here the relationship of each characteristic is calculated separately with the CT results. After MVA, the ORs for the male sex, age > 59 years, former smoker, non-smoker, abnormal radiograph, DLCO < 80%, and FEV1/FVC < 70% in the robust interval of positively associated values. In addition, FVC < 80% and pre-existing respiratory disease are robustly adversely correlated with an abnormal CT outcome. The ORs for the remaining characteristics are in the weak correlation range. Male sex, age > 59 years, and abnormal radiography were statistically significant.

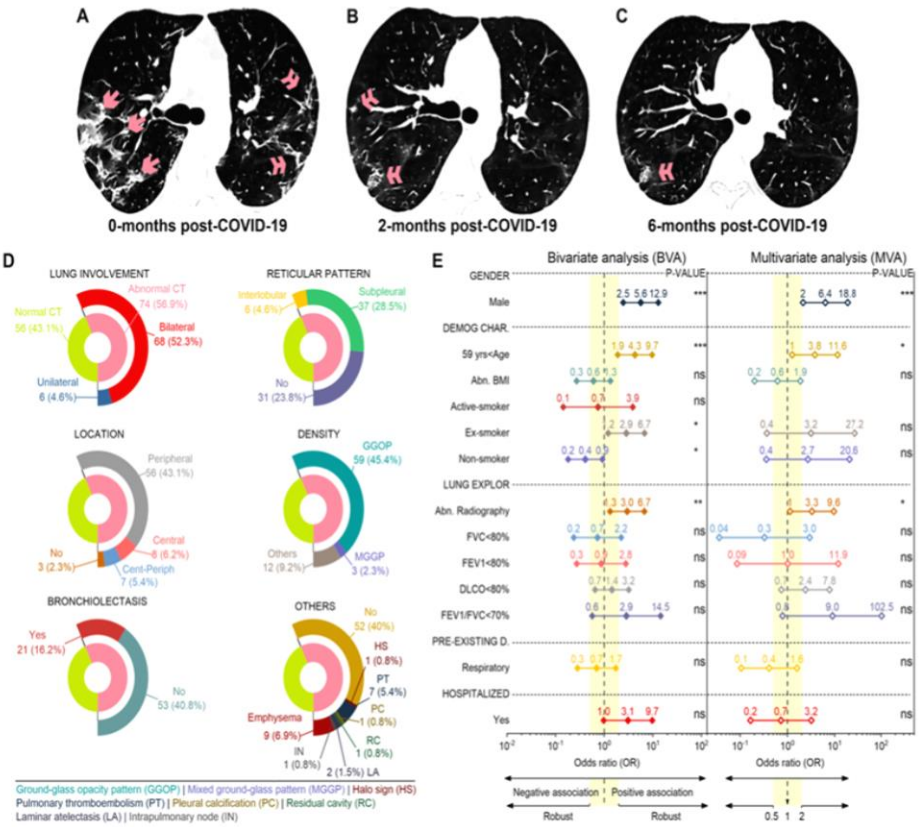

Figure 5. Evolution of CT scans at the same level for one patient at 0 (**A**), 2 (**B**), and 6 (**C**) months post-COVID-19. The predominant imaging pattern was the GGOs (single arrow) and reticular (double arrow). (**D**) Chest CT scan findings in all patients at the 6-month follow-up. (**E**) Forest plot of the odds ratio and its CIs (95%) values for the bivariate and multivariate analysis. Relationship between the PCS features with the normal/abnormal chest CT outcome.

Discussion

We have presented quantitative analyses demonstrating the presence of PCS in patients at 6-month follow-up. We started with a descriptive analysis of the clinical, mental health, physical examination, and laboratory indices findings. We have shown that the most prevalent characteristics are: dyspnea, fatigue, dry cough, loss of taste/smell, headache, hair loss, emotional affectation, depression, and cognitive deficit for clinical and mental health findings; and hemoglobin, lymphocytes, neutrophils, platelets, total bilirubin, creatine, ALT total protein, glucose, LDH, ferritin, and D-dimer for abnormal laboratory indices. This agrees with findings from previous long-term follow-up studies of SARS[11,13] and MERS[31,32]. In addition, we have shown that fatigue, arthralgia, fever, hair loss, emotional affectation, depression, cognitive deficit, hemoglobin, total bilirubin, and ferritin associated with PCS depend on the patient's gender. In particular, the female sex is favorable for the persistence of symptomatologies—matching prior findings[33].

At 6-months of follow-up, we found that the previous respiratory diseases and hospitalization status are strongly associated with specific demographic characteristics, clinical symptoms, mental health, and pulmonary function tests based on single and multiple PCS features. We found that PFTs were affected in patients with or without PRD—whether hospitalized or not-during the acute viral process and pulmonary involvement with restrictive and obstructive patterns and impaired diffusion capacity. The most frequent parameter to be highlighted was the diffusion capacity impairment. Its decrease may suggest an incipient DILD or the presence of pulmonary vascular abnormalities secondary to COVID-19. The bivariate analysis demonstrated the robust statistically significant association of patients with previous respiratory diseases with the following essential features: abnormal BMI, as a demographic characteristic, dyspnea and fatigue as clinical features, emotional affectation and depression as psychological complications, and impaired of FEV1, DLCO, and the FEV1/FVC ratio (i.e., positive diagnostic of obstructive and restrictive lung disease). However, after using more than one independent PCS feature, only the abnormal BMI, ex-smoker, and dyspnea had a robust statistically significant

association to patients with PRD. This result implies that patients with PRD with one or more PCS features need to be monitored on a long-term follow-up basis. The following characteristics were obtained from the bivariate analysis for hospitalization status with a robust association and significant relationships: male sex, older than 59 years old, non-health worker, and venous thrombosis. Nevertheless, after the multivariate analysis, the robustness of the association for the predictors increased—except for the age > 59 years, adding fatigue as a new significant characteristic. The influential negative association of thromboembolic events—OR ≪ 1—and fatigue suggests that patients without hospitalization also need long-term follow-up.

Our findings show the positive association between abnormal BMI and hospitalization patients for COVID-19—matching previous results[34], adding the new association with the presence of PRD. Regards to this, there is a debate on changing the relationship on BMI fluctuation of patients with overweight or obesity hospitalized for COVID-19 during their follow-up. We claim that nutritional management strategies[35,36] during hospitalization and after discharge must be implemented to improve short- and long-term follow-up outcomes considering the comorbidities—as Di Filippo and colleagues proposed[34].

The major strength of our study is the long-term follow-up of patients with the examination of all patients reported at 2 and 6 months, including hospitalized and non-hospitalized patients. This study is the first to present single and multiple characterizations of the long-term sequelae of COVID-19. Moreover, our study is, to date, one of the most detailed and most prolonged follow-up studies of post-COVID-19 patients. However, the greater willingness of symptomatic patients to participate in a follow-up study is a possible biasing factor—as in all observational studies. The study findings may be limited due to the single-center, nonblinded, and nonrandomized design. We understand this potential localization bias.

Conclusions

At 6-months follow-up, PCS characteristics fatigue, arthralgia, fever,breathlessness, emotional disturbance, depression, cognitive deficit, hemoglobin, total bilirubin, and ferritin are correlated with the gender of the patient. Patients with previous respiratory diseases and abnormal body mass index, ex-smoker, and dyspnea had a robust statistically significant association. Non-hospitalized patients may suffer more severe thromboembolic events and fatigue than hospitalized patients. Functional lung tests are good predictors of chest CT imaging abnormalities in elderly patients with PCS.

The preliminary study presented here can be extended in several ways. First, the study can be prolonged to 12, 24, and 36 months of follow-up. This will enable us to study the long-term effects of PCS and define different degrees of severity. Second, adding new variables to the study will allow us to create models to predict the most frequent symptoms for medical treatments. In fact, we are working to improve our study in these directions.

Materials and methods

Deffiniton of post-COVID-19 syndrome (PCS). Signs and symptoms that develop during or after an infection consistent with COVID-19, present for more than 12 weeks, and are not attributable to alternative diagnoses—following the guideline of the National Institute for Health and Care Excellence (NICE), the Scottish Intercollegiate Guidelines Network (SIGN), and the Royal College of General Practitioners (RCGP)[17].

Study population. The prospective cohort study was conducted at the University Hospital Virgen de la Nieves, one of the hospitals assigned for patients with COVID-19 in Granada, Spain. Two visits were scheduled in the follow-up period from May to October 2020. A total of 372 patients—with 217 included and 115 excluded—were referred 2-months after diagnosis of COVID-19 for the first follow-up consultation (FFuC). The 217 patients included in the FFuC were referred to the second follow-up consultation (SFuC) at 6-months after initial diagnosis—with 207 included and 10 excluded. For COVID-19 detection, was used the RT-PCR from the upper respiratory tract (nasopharyngeal and oropharyngeal swab) or lower respiratory tract (sputum collection) and antibody serology (IgM and IgG) by ELISA. The study timeline, flowchart, and follow-up consultation procedures are shown in Fig. 1. The study was conducted following the requirements of the Declaration of Helsinki and the Spanish Data Protection Act of 15/1999. Following the Declaration of Helsinki, written informed consent was obtained from all patients, and local ethics committees approved the study.

The study population consisted of patients aged > 14 years, diagnosis of infection confirmed according to international recommendations and signing the informed consent. The exclusion criteria were: suspected cases of SARS-CoV-2, immunosuppressed patients with therapeutic limitation due to terminal pathology, and those who refused to participate (see Fig. 1). The age, sex, BMI, toxic habits, profession, family members with confirmed or suspected COVID-19, and need or not of hospital admission were considered. See Table 2, which summarizes the characteristics of the patients included in the study.

Data collected in follow-up consultations. The clinical features studied at each follow-up visit were: dry and wet cough, dyspnea, fatigue, muscle weakness, musculoskeletal involvement, chest pain, palpitations, fever, sweating, intestinal disorders, post-COVID-19 arterial hypertension, otorhinolaryngologic symptoms, ocular symptoms, neurological manifestations, decreased sexual appetite, cutaneous manifestations and other expressed symptoms—thromboembolic events. Also, each consultation included mental abnormalities, physical examination, and laboratory indices (see Tables 1, 2).

In all patients, a chest radiograph was performed in posterior-anterior and lateral projection at the FFuC. Then, a complementary study with HRCT was requested if there were any abnormal findings on the X-ray. HRCTs were evaluated by radiologist specialists and one pulmonologist and reported according to the Spanish

Test in the study population (units)	Reference ranges	
	Laboratory-UH-VN	References[39,42,43]
Biochemical		
Glucosa (mmol/L)	4.16–6.38	3.9–6.1
Total bilirubin (µmol/L)	5.13–20.52	0–26
Creatinine (µmol/L)	59.23–106.08	57–111
Alanine aminotransferase (ALT, SGPT) (U/L)	7–34	10–40
Aspartate aminotransaminase (AST, SGOT) U/L	10–35	10–40
Lactato deshidrogenasa (LDH), U/L	0–247	0–245
Serum ferritin (ng/mL)	10–120	21–274.66
Total protein (g/dL)	6.6–8.3	6.5–8.5
Hematologic		
Haemoglobin (g/dL)	12–15.6	13–17.5
Leukocytes (10^9 L)	3.9–10.2	3.5–9.5
Neutrophils (%)	42–77	50–70
Lymphocytes (%)	20–44	30–45
Lymphocytes ($\times 10^9$ L)	1.1–4.5	1.1–3.2
Platelets ($\times 10^9$ L)	130–370	125–359
Coagulation function		
D-Dimer (µg/L)	0–0.5	0–1

Table 1. Comparative table of the laboratory reference ranges considered normal in the adult population according to the international system and the provincial area of Granada. Laboratory reference ranges and characteristics of the patients included in the study.

Society of Medical Radiology (SERAM) recommendations, the international standard nomenclature defined by the Fleischner Society glossary and existing publications until now. Each imaging test was analyzed considering: density (ground glass, consolidation or mixed), lung involvement (unilateral or bilateral), location (central, peripheral or mixed), presence of reticular pattern or interstitial lesions of pulmonary parenchymal (subpleural or interlobular), and the percentage of lung extension involved < 20%, 20–50% and > 50%—according to the lung fields involved (see Table 2).

At the SFuC, each patient underwent forced spirometry, lung volume, diffuse capacity, and the 6MWT. The functional exploration was carried out with experienced personnel with the equipment of MasterScreen Body, brand Jaeger, Germany. According to American Thoracic Society and Spanish regulations, the reference values for the Mediterranean population and acceptability criteria. Pulmonary parameters included FEV1, FVC, the FEV1/FVC ratio, RV, TLC, DLCO, and KCO.

Statistical analysis. Descriptive analysis was carried out using number (%), median, and its interquartile range (IQR)—combining box plots and density plots, i.e., violin plots—for categorical and continuous variables, respectively[39,40]. Discrepancies in the patient characteristic distributions by sub-groups of outcomes are presented as differences with 95% confidence intervals (CIs). The Mann–Whitney U test—for nonnormal distributed continuous data, χ^2 test, or Fisher's exact was used to compare clinical features, physical examination, mental health, and laboratory indices between males and females at the first and second follow-up consultations.

Bivariate and multivariate analysis—using the maximum likelihood estimation to obtain the coefficients and the of Hosmer–Lemeshow goodness-of-fit test for the mode—was carried out to compute the odds ratios (ORs) and 95% CIs to explore the association with the following features: at least one pre-existing respiratory disease, hospitalized patients, and an abnormal chest CT finding[41]. The degree of association of the PCS is defined according to the OR value (robust or not). Data cleaning and analysis using logistic regression models were implemented in Python 3.7. The tests were two-sided, and a p value less than $\alpha = 0.05$ was considered statistically significant.

Characteristics (N = 217)
Age (years), median (IQR)
Gender, (N (%))
Female and Male
BMI (kg/m2)
Smoking history, (N° (%)): Cumulative tobacco burden index (ICAT), Current, Former and Nonsmoker
Healthcare professional (N° (%))
Need or not of hospital admission (N° (%))
First follow-up consultations (N = 217)
Clinical features
Asymptomatic (N° (%))
Any one of the following symptoms N° (%): Dyspnea, Fatigue, Cough (dry and wet), Muscle weakness, Arthralgia or Myalgia, Chest pain, Palpitations, Fever (temperature ≥ 37.3 °C), Sweating, N° (%)
Intestinal disorders (N° (%)): Nausea, Vomiting, Diarrhoea
Post covid arterial hypertension, N° (%)
Otorhinolaryngologic symptoms (N° (%)): Rhinorrhea, difficulty to swallow, tinnitus, nasal dryness, dysphonia, and hearing loss
Loss of taste or smell, dizziness, and gait instability (N° (%))
Ocular symptoms (N° (%))
Epiphora and reduced visual acuity (N° (%))
Neurological Manifestations (N° (%)): Polyneuro/myopathy, Headaches, Cognitive deficits
Erectile dysfunction and decreased sexual apetite (N° (%))
Cutaneous manifestations (N° (%)): Skin rash, Rash skin eruptions, Hair loss
Thromboembolic events (N° (%)) and types thromboembolic events
Mental health
Emotional affectation, Depression, and Sleep disturbance (N° (%))
Physical examination
Lung auscultation, crackles, Sat02, nail clubbing (N° (%))
Abnormal finding on X-ray, (N° (%))
Laboratory finding (Table 1)
Second follow-up consultations (N = 207)
Clinical features, mental health, physical examination, X-ray, and laboratory tests as in the FFuC
Pre-existing respiratory disease (PRD) (N° (%))
Pulmonary function
FVC < 80%, FEV1 < 80%, FEV1/FVC < 70%, TLC < 80%, VR < 65%, DLCO < 80% and KCO < 80% (% of predicted)
6MWT
Distance-meters, median (IQR) and oxygen saturation, median (Initial, Final, and Average) (IQR)
Chest CT
Density (N° (%)): Mixed pattern, Consolidation, and Ground-glass
Location (N° (%)): Peripheral Central and Mixed
Subpleural reticular pattern (N° (%)): Interlocular septal thickening and thickening of the adjacent pleura
Lung involvement (N° (%)): Unilateral and Bilateral
Bronchiectasis (N° (%))
Others findings of CT (N° (%))

Table 2. Summary of the characteristics of the patients included in the study.

Data availability

Currently, the third follow-up consultation (one year after the disease) is being collected, which means that data cannot be shared. Once the data analysis process for each follow-up process is completed, the data and the implemented code could be shared on an internet hosting, such as GitHub repository.

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Author contributions

C.M.N. proposed and directed the research with help from B.M.J.R., J.G.F., and R.F.; M.I.S.P., and M.J.M.L. carried out the measurement and data collection of the pulmonary function tests; B.M.J.R. collected the demographic, clinical, and psychological data in follow-up consultations supported by P.I.G.F. and E.M.R.U.; G.L.M. and B.M.J.R. performed the acquisition and processing of HRCT images; J.G.F. undertook the processing of the laboratory indices; R.F. and B.M.J.R. conducted the data analysis in Python; C.M.N., B.M.J.R., J.G.F., and R.F. wrote the manuscript; and all authors contributed to discussions.

Competing interests

The authors declare no competing interests.

Additional information

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11.2 Artículo 2

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Article

[^{18}F]FDG PET/CT in Short-Term Complications of COVID-19: Metabolic Markers of Persistent Inflammation and Impaired Respiratory Function

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Abstract: SARS-CoV-2 virus infects organs other than the lung, such as mediastinal lymph nodes, spleen, and liver, but, to date, metabolic imaging studies obtained in short-term follow-ups of patients hospitalized with severe COVID-19 infection are rare. Our objective was to evaluate the usefulness of [^{18}F]FDG-PET/CT in the short-term follow-up of patients admitted for COVID-19 pneumonia and to explore the association of the findings with clinical prognostic markers. The prospective study included 20 patients with COVID-19 pneumonia (November 2020–March 2021). Clinical and laboratory test findings were gathered at admission, 48–72 h post-admission, and 2–3 months post-discharge, when [^{18}F]FDG-PET/CT and respiratory function tests were performed. Lung volumes, spirometry, lung diffusion capacity for carbon monoxide (DLCO), and respiratory muscle strength were measured. Volumetric [^{18}F]FDG-PET/CT results were correlated with laboratory and respiratory parameters. Eleven [^{18}F]FDG-PET/CT (55%) were positive, with hypermetabolic mediastinal lymphadenopathy in 90.9%. Mediastinal lesion's SUVpeak was correlated with white cells' count. Eleven (55%) patients had impaired respiratory function, including reduced DLCO (35%). SUVpeak was correlated with %predicted-DLCO. TLG was negatively correlated with %predicted-DLCO and TLC. In the short-term follow-up of patients hospitalized for COVID-19 pneumonia, [^{18}F]FDG-PET/CT findings revealed significant detectable inflammation in lungs and mediastinal lymph nodes that correlated with pulmonary function impairment in more than half of the patients.

Keywords: COVID-19; [^{18}F]FDG-PET/CT; respiratory function test; inflammatory; complications; SARS-CoV-2

1. Introduction

There is growing interest in the diagnosis, prognosis, and optimal clinical management of the sequelae of acute COVID-19 infection.

In the acute phase of infection, the epidemiology, clinical characteristics, results of standard clinical laboratory tests, lung CT appearance, treatment strategies, and outcomes in patients with COVID-19 have been reported in previous studies [1]. Imaging techniques, especially high-resolution computed tomography (HRCT), have demonstrated a relevant

diagnostic role [2], and multiple studies have been published on radiological findings in patients with COVID-19 pneumonia, especially during the acute phase and, more recently, over the short and medium terms [2,3].

The SARS-CoV-2 virus has been shown to infect organs other than the lung, such as the mediastinal lymph nodes, spleen, and liver, quantitative case studies in patients with COVID-19 are rare [3,4]. Such information can be obtained through the use of [^{18}F]- 2-Fluoro-2-Deoxy-Glucose ([^{18}F]FDG) positron emission tomography/computed tomography (PET/CT), which is commonly used to assess inflammatory and infectious lung diseases [5].

The complementary functional information provided by [^{18}F]FDG-PET/CT, which has been shown to be useful for diagnosing inflammatory and infectious lung diseases, estimating their severity, monitoring their evolution, and evaluating therapeutic response [4,5], can help elucidate the pathophysiological mechanisms of COVID-19. The value of [^{18}F]FDG-PET/CT has been reported in patients with respiratory infections caused by other coronaviruses, such as MERS-CoV and SARS-CoV [6,7], as well as in patients with acute COVID-19 infection [4,8].

The [^{18}F]FDG-PET/CT studies of asymptomatic cancer patients described the incidental detection of interstitial pneumonia compatible with possible acute SARS-CoV-2 infection [7], and researchers have begun to examine the potential role of [^{18}F]FDG-PET/CT in its diagnosis and treatment [8]. As well as visual interpretation by an experienced specialist, [^{18}F]FDG-PET/CT also offers a semiquantitative approach to glycemic metabolism and, therefore, the intensity of inflammatory activity. Besides the standardized uptake value (SUV), recent studies in oncology have yielded additional parameters such as the metabolic tumor volume (MTV) and total lesion glycolysis (TLG) [9], which could be used to estimate inflammatory activity in lungs or extrapulmonary organs, especially lymph nodes. Studies of noncritical hospitalized patients have highlighted the possible relevance of lymph node hypermetabolism, quantified by the maximum SUV (SUVmax) in PET images, proposing that the highest SUVmax values for lesions and lymph nodes may indicate an increased severity of the infection and may predict a poor prognosis [3,4].

With this background, we hypothesized that [^{18}F]FDG-PET/CT could be useful to characterize pulmonary sequelae of COVID-19 infection. The objective of this study was to evaluate the usefulness of [^{18}F]FDG-PET/CT in the short-term follow-up of patients admitted for COVID-19 pneumonia and to explore the association of findings with clinical prognostic markers

2. Materials and Methods

2.1. Patients

This prospective, longitudinal, observational study enrolled consecutive COVID-19 patients at their follow-up visit 1–2 months after discharge from a third-level hospital between 27 November 2020 to 1 March 2021.

Study inclusion criteria were confirmation of COVID-19 in accordance with WHO guidelines [10] by a positive RT-PCR result for nasopharyngeal swabs, hospital admission between November 2020 to March 2021 (dates of "third wave" in Spain), and findings of ground-glass opacity or consolidation on chest HRCT scan or X-ray at admission. Exclusion criteria were age under 18 years, absence of microbiological confirmation of COVID-19 infection, history or presence of pulmonary fibrosis, active or uncontrolled COVID-19 infection at the time of the [^{18}F]FDG-PET/CT study, history or suspicion of oncological disease, pregnancy, and inability to sign informed consent.

The study was approved by the local Research Ethics Committee, and written, informed consent was obtained from all participants. Personal protective equipment was available for all staff, and COVID-19 infection prevention guidelines were always rigorously followed [11].

tions [16,17]. They characterized the density (alveolar, ground glass, or mixed), distribution (central, peripheral, or diffuse), location (unilateral or bilateral), and extent (unilobar or multilobar).

2.7. Statistical Analysis

All measurements for each participant were independently conducted by two nuclear medicine physicians, considering the mean value in statistical analyses. Absolute numbers and percentages were calculated for categorical variables and means with standard deviation (SD) for continuous variables. For comparisons of quantitative data between the positive and negative PET groups, the Student's *t*-test was applied when the distribution was normal and the Mann–Whitney U test when it was not. Associations with categorical variables were evaluated by constructing contingency tables, applying the chi-square test for individual comparisons and Fisher's exact test for multiple comparisons. Volumetric [^{18}F]FDG-PET/CT results were correlated with laboratory test results and respiratory function parameters by using Spearman's rank correlation coefficient. IBM SPSS version 15.0 (IBM Corp, Armonk, NY, USA) and R software were used for statistical analyses. A $p \leq 0.05$ was considered significant in all tests.

3. Results

The study included 20 patients (60% males) with a mean age of 55.85 ± 9.28 years admitted for pneumonia and/or respiratory failure between 27 November 2020 and 1 March 2021 (during the "third wave" of COVID-19 in Spain). The mean hospital stay was 16.70 ± 11.99 days. Table 1 summarizes the baseline characteristics of the patients.

Table 1. Baseline clinical characteristics and risk factors of patients.

Clinical Characteristics (*n*)	Mean ± SD or *n* (%)
Age (years)	55.85 ± 9.28
Gender (Male)	12 (60)
BMI (kg/m^2)	34.11 ± 7.23
Comorbidities	
Former or current smoking habit	2 (10)
Hypertension	5 (25)
Diabetes	3 (15)
Hyperlipidemia	2 (10)
Atrial fibrillation	2 (10)
Asthma	3 (15)
Charlson Comorbidity index	1.60 ± 1.14
Charlson Comorbidity index ≥ 2	9 (45)
Clinical characteristics at admission	
Fever	17 (85)
Dyspnea	15 (75)
Irritative cough	16 (80)
Fatigue	14 (70)
Myalgia	11 (55)
Anosmia/Ageusia	2 (10)
Digestive symptoms	9 (45)
Headache	3 (15)
ARDS (PaO_2/FIO_2 < 300 mmHg)	14 (70)
Blood oxygen saturation	90.90 ± 5.33

2.2. Clinical Information and Laboratory Test Results

For all patients, data were gathered from electronic medical records, including the results of clinical and laboratory tests at admission, at 48–72 h post-admission, and at the follow-up PET/CT examination. Analytical data included complete blood count, standard blood biochemistry, acute phase reactants, coagulation status [12], and neutrophil/lymphocyte ratios (NLRs). All patients underwent RT-PCR for nucleic acid testing of SARS-CoV-2.

2.3. Respiratory Function Tests

Respiratory function tests were performed at 2–3 months after hospital discharge. Spirometry results (in mL and % predicted) were obtained for forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and FEV1/FVC ratio. Body plethysmography was used to measure the residual volume (RV, in mL and % predicted), and total lung capacity (TLC, in mL and % predicted). The diffusing capacity of the lungs for carbon monoxide (DLCO) and the CO transfer coefficient (KCO) were expressed in absolute numbers and as % predicted. The results of the 6-min walk test (TM6M) were expressed as distance (in m) and % oxygen saturation at start and finish. Specifically trained personnel carried out functional tests using MasterScreen Body equipment (Jaeger, Hoechberg, Germany), considering reference values for the Mediterranean population and acceptability criteria established by European and Spanish regulations [13,14].

2.4. PET/CT Data Acquisition

After two consecutive negative RT-PCR test results for SARS-CoV-2 nucleic acid, confirming that patients were no longer infected, patients underwent [^{18}F]FDG-PET/CT imaging (Siemens Biograph Vision 600 PET/CT, Siemens Healthcare, Erlangen, Germany), always performed within 2–3 months after discharge from hospital. The test protocol was based on international recommendations [15]. Patients were administered intravenously with the radiopharmaceutical (3.7–4.81 MBq/kg) at rest after fasting for at least 6 h with adequate hydration as long as their capillary blood glucose level was below 6.8 mmol/L. Image acquisition (whole body in 3D) started at 50–60 min post-injection with the acquisition of a topogram (50 mA, 120 kV), followed by helical CT without contrast (170 mA, 120 kV) and the acquisition of PET images with coverage from skull base to mid-thigh.

2.5. PET/CT Image Interpretation

The [^{18}F]FDG-PET/CT and chest CT images were independently analyzed by two nuclear medicine physicians (E.M.T.I. and M.G.M.) with a great deal of experience in the interpretation of cardiothoracic images, using syngo.via version VB40B software (Siemens Healthcare, Erlangen, Germany). They were blinded to the biological and clinical data of patients. Discrepancies in interpretations were resolved by consensus with a third expert nuclear medicine physician (A.R.F.).

The [^{18}F]PET/CT data were transferred to a computer workstation (syngo.via) for the co-registration of PET and CT images. Regions of interest (ROIs) were drawn on CT images of lungs around areas with evident loss of aeration and adjacent areas of normal appearance. ROIs were also drawn on CT images of mediastinal lymph nodes. The ROIs drawn on the CT images of each patient were transferred to the co-registered PET images and the amount of [^{18}F]FDG pathological uptake was calculated for each ROI, determining maximum, peak, and minimum SUVs, normalized by body weight (SUVmax, SUVpeak, and SUVmin, respectively) and lean body mass (SUL); metabolic tumoral volume (MTV; volume of pixels in the ROI with SUVmax >40%); and total lesion glycolysis (TLG; MTV multiplied by SUVmean).

2.6. Chest CT and X-ray Image Interpretation

Upon their diagnosis, all patients underwent chest X-ray in posterior-anterior and lateral projections, reported by specialist radiologists according to current recommenda-

Table 1. *Cont.*

Clinical Characteristics (*n*)	Mean ± SD or *n* (%)
Laboratory test results at admission	
Hemoglobin (g/dL)	14.86 ± 1.84
White blood cell (count $\times 10^3/\mu L$)	7.68 ± 3.11
Neutrophil (count $\times 10^3/\mu L$)	6.28 ± 3.16
Lymphocyte (count $\times 10^3/\mu L$)	0.99 ± 0.57
NLR	8.36 ± 5.86
Platelet (count $\times 10^3/\mu L$)	206.65 ± 54.49
Ferritin (ng/mL)	1327.81 ± 1402.58
C-reactive protein (mg/L)	81.20 ± 54.61
LDH (U/L)	398.15 ± 113.80
AST(U/L)	49.25 ± 38.60
ALT(U/L)	48.30 ± 46.36
Albumin (g/dL)	3.92 ± 0.50
D-dimer (mg/L)	0.73 ± 0.52
Characteristics of Hospitalization	
Hospital stay (days)	16.70 ± 11.99
Pneumonia (chest X-ray)	19 (95)
ICU admission	10 (50)
Invasive mechanical ventilation	5 (25)
Bolus therapy with glucocorticoid	14 (60)
Antiviral therapy	5 (25)
Selective inhibitors of pro-inflammatory cytokines	6 (30)

Continuous variables are presented as means ± standard deviation (SD) and categorical variables as frequencies (percentages). ARDS: acute respiratory distress syndrome; AST: aspartate aminotransferase; ALT: alanine transaminase; NLR: neutrophil/lymphocyte ratio; PCT: procalcitonin; NT-proBNP: N terminal pro-B-type natriuretic peptide; LDH: lactate dehydrogenase; ICU: intensive care unit.

The main symptom at admission was fever in 17/20 patients (85%), followed by irritative cough in 16 (80%), dyspnea in 15 (75%), fatigue in 14 (70%), and ageusia and/or anosmia in 2 patients (10%). Chest X-ray findings compatible with pneumonia were observed in 19 patients (95%), being multilobar in 18 (97.4%) and unilobar in 1 (5.3%). The radiological pattern was alveolar in three patients (15.8%), ground glass in seven (36.8%), and mixed in the remaining nine (47.4%). The main associated complication during hospitalization was respiratory distress in 14 (70%) patients; admission to the intensive care unit (ICU) was required for seven (35%) of these patients and invasive mechanical ventilation in five (25%). All patients were treated with corticosteroids, administered as a bolus in 14 patients (70%). Five patients (25%) received antiviral treatment and another six (30%) were treated with selective inhibitors of pro-inflammatory cytokines (five with tocilizumab and one with anakinra). Finally, three patients (15%) required home oxygen therapy at discharge.

3.1. The [^{18}F]FDG-PET/CT Findings

The mean time from hospital discharge to [^{18}F]FDG-PET/CT study was 58.85 ± 13.67 days. The result was positive in 11 patients (55%) and negative in 9 (45%). The main finding was hypermetabolic lymphadenopathy in the mediastinum, observed in 10 (90.9%) of the [^{18}F]FDG-PET/CT-positive patients (Figure 1).

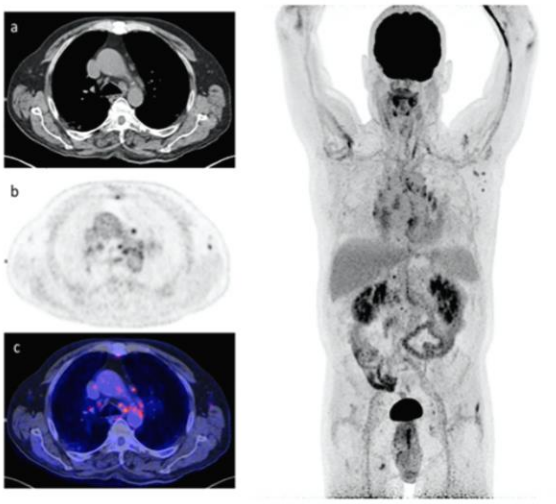

Figure 1. A 64-year man admitted for multilobar pneumonia caused by SARS-CoV-2. The [^{18}F]FDG-PET/CT at 3 months after symptom onset shows increased [^{18}F]FDG uptake in residual pulmonary lesions (TLG 124,11) and mediastinum lymph node (SUVpeak 1,73). Pulmonary function tests evidenced severe pulmonary diffusion impairment, with a diffusing capacity of the lungs for carbon monoxide 41% of the predicted value. Left: (**a**) CT transverse slice, (**b**) [^{18}F]FDG-PET slice, and (**c**) fused [^{18}F]FDG-PET and CT images. Right: whole-body, maximal intensity projection image, displaying mediastinal lymph nodes with [^{18}F]FDG uptake.

Patients with positive and negative [^{18}F]FDG-PET/CT results significantly differed in age (59.82 ± 8.52 vs. 51.00 ± 8.09 years, respectively, $p = 0.03$), Charlson index score ≥1 (66.7 vs. 100%, $p = 0.038$), presence of fatigue (90.9 vs. 44.4%, $p = 0.024$) and respiratory distress (90.9 vs. 44.4%, $p = 0.024$), hemoglobin levels (13.41 ± 1.91 vs. 15.24 ± 1.58 g/dL, $p = 0.041$), and lymphocyte count (1.78 ± 0.53 vs. 2.47 ± 0.49 × 10^3/μL, $p = 0.011$) at 2–3 months post-discharge (Figure 2).

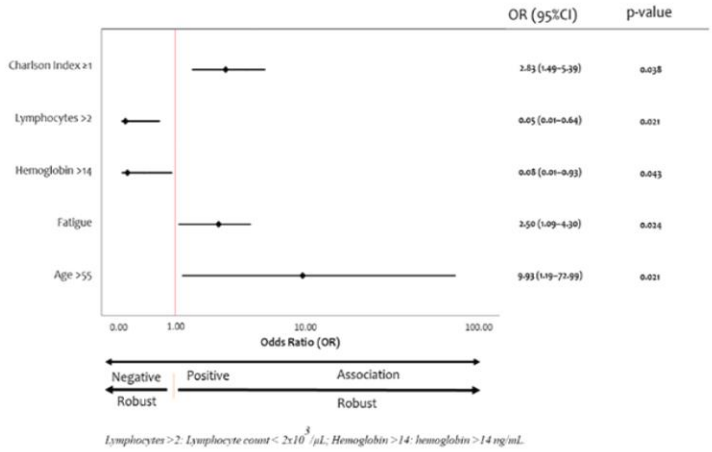

Figure 2. Factors associated with [^{18}F]FDG-PET/CT positive. Forest plot with odds ratios shown by closed circles and 95% confidence intervals by whiskers.

3.2. Correlation of Volumetric [^{18}F]FDG-PET/CT Parameters with Laboratory Test Results

Table 2 exhibits associations observed between volumetric [^{18}F]FDG-PET/CT results and analytical findings at admission, during the hospital stay, and at 2–3 months post-discharge.

Table 2. Bivariate correlations of volumetric [^{18}F]FDG PET/CT parameters with laboratory parameters at admission, during hospital stay, and at 2–3 months post-discharge (short-term follow-up).

Variable		SUVPeak		Pulmonary TLG	
		Spearman's rho	*p*-Value	Spearman's rho	*p*-Value
Admission	Hemoglobin (g/dL)	−0.664	0.026		
	Neutrophil count	−0.764	0.006		
	Lymphocyte count	0.636	0.035		
	NLR	−0.664	0.026		
Hospital stay	Neutrophil count	−0.700	0.016		
	Lymphocyte count	0.618	0.043		
	NLR	−0.627	0.039		
	IL-6			0.624	0.010
	C-reactive protein			0.618	0.004
	PCT			0.570	0.049
	LDH			0.445	0.049
	Troponin			0.883	0.002
	Fibrinogen			0.635	0.015
	D-dimer			0.674	0.001
Short-term follow-up	Neutrophil count	−0.679	0.022		
	Lymphocyte count	0.791	0.004		
	NLR	−0.727	0.011		

IL-6: interleukin 6; LDH: lactate dehydrogenase; NLR: neutrophil/lymphocyte ratio; PCT: procalcitonin.

At admission, a significant correlation was found between the SUVpeak of the target lesion in the mediastinum and the hemoglobin level (r = 0.615, $p = 0.044$), leukocyte count (rho = −0.664, $p = 0.026$), neutrophil count (rho = −0.764, $p = 0.006$), lymphocyte count (rho = 0.636, $p = 0.035$), and NLR (rho = −0.664, $p = 0.026$). In addition, the TLG in lung parenchyma was significantly correlated with C-reactive protein (CRP) (rho = 0.558, $p = 0.011$), procalcitonin (rho = 0.611, $p = 0.035$), fibrinogen (rho = 0.472, $p = 0.041$), and blood glucose (rho = 0.517, $p = 0.020$) levels at hospital admission.

During hospitalization, the SUVpeak of the target lesion was again significantly correlated with neutrophil count (rho = −0.700, $p = 0.016$), lymphocyte count (rho = 0.618, $p = 0.043$), and NLR (rho = −0.627, $p = 0.039$). Furthermore, pulmonary TLG was significantly correlated with IL-6 (rho = 0.624, $p = 0.010$), CRP (rho = 0.618, $p = 0.004$), procalcitonin (rho = 0.570, $p = 0.042$), LDH (rho = 0.445, $p = 0.049$), troponin (rho = 0.883, $p = 0.002$), fibrinogen (rho = 0.635, $p = 0.015$), and D-dimer (rho = 0.674, $p = 0.001$) levels and with the neutrophil count (rho = 0.615, $p = 0.044$), lymphocyte count (rho = −0.615, $p = 0.004$), and NLR (rho = 0.558, $p = 0.011$) during the hospital stay.

At the follow-up at 2–3 months, the SUVpeak was significantly correlated with neutrophil count (rho = −0.679, $p = 0.022$), lymphocyte count (rho = 0.791, $p = 0.004$), and NLR (rho = −0.727, $p = 0.011$). No significant correlation was found between pulmonary TLG and any analytical parameter under study (Figure 3).

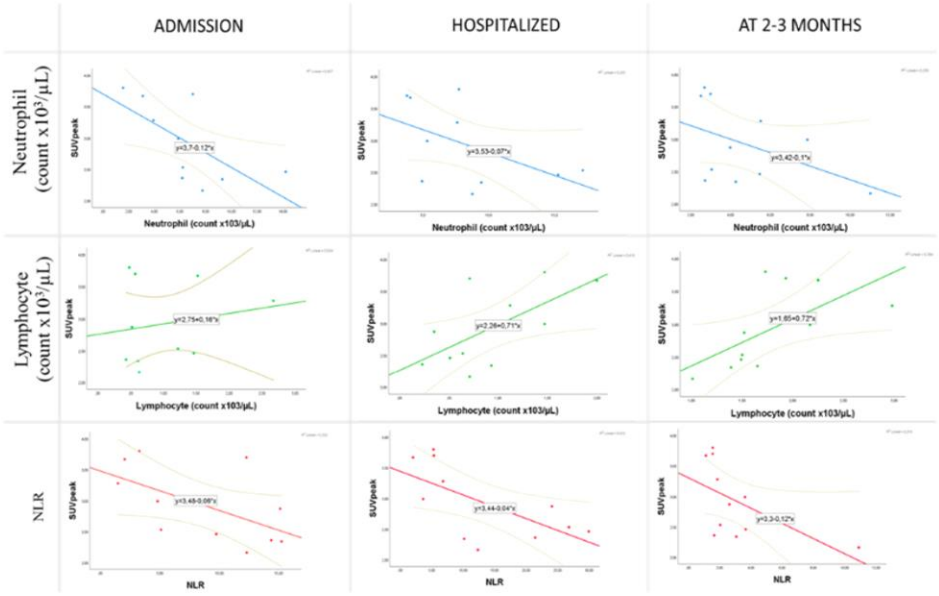

Figure 3. Scatter plot matrix. Correlation of the SUVpeak of the target lesion with neutrophil and lymphocyte counts and NLR at admission, during hospital stay, and at 2–3 months post-discharge.

3.3. *Correlation of Volumetric [18F]FDG-PET/CT Parameters with Respiratory Function Parameters*

Eleven (55%) of the 20 patients had impaired respiratory function. Percentage predicted values were <80% for FVC in 20% of patients, <80% for FEV1 in 15%, <70% for FEV1/FVC in 5%, <80% for TLC in 20%, <80 for DLCO in 35%, <80% for KCO in 25%, and <65% for VR in 5%. Saturation was ≥4% lower at the finish versus start of the walk test in four patients (20%), and the distance was <400 m in three (15%).

Volumetric [18F]FDG-PET/CT parameters were related to respiratory function test results obtained at 2–3 months post-discharge (Figure 4). The SUVpeak of the target lesion in the mediastinum was significantly and positively correlated with % predicted DLCO (rho = 0.782, $p = 0.008$), KCO (rho = 0.721, $p = 0.019$), and RV (rho = 0.636, $p = 0.048$) values. Pulmonary TLG was significantly and negatively correlated with % predicted DLCO (rho = −0.628, $p = 0.005$), KCO (rho = −0.564, $p = 0.014$), TLC (rho = −0.532, $p = 0.023$), and RV (rho = −0.554, $p = 0.017$) values. (Table 3).

Table 3. Bivariate correlations of volumetric [18F]FDG PET/CT parameters and respiratory function parameters in the short-term follow-up.

Variable	SUVpeak		Pulmonary TLG	
	Spearman's rho	*p*-Value	Spearman's rho	*p*-Value
DLCO% pred	0.782	0.008	−0.628	0.005
KCO% pred	0.721	0.019	−0.564	0.014
TLC% pred	0.467	0.174	−0.532	0.023
RV% pred	0.636	0.048	−0.554	0.017

DLCO: diffusing capacity of the lungs for carbon monoxide; KCO: CO transfer coefficient; RV: residual volume; TLC: total lung capacity.

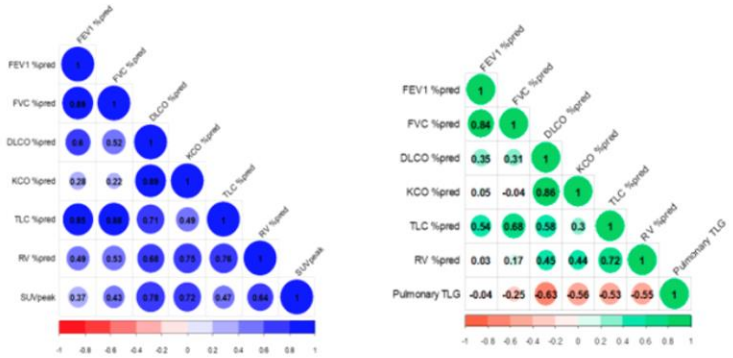

Figure 4. Correlogram showing the association of respiratory function test results with the SUVpeak of the target lesion and pulmonary TLG.

4. Discussion

In this study, [^{18}F]FDG-PET/CT was used to measure the metabolism of lungs and other organs in the short–medium follow-up of patients admitted to hospital for pneumonia or respiratory failure due to COVID-19 infection. Despite testing negative for the infection in two successive RT-PCR tests of nasopharyngeal swabs, more than half of the patients showed increased metabolic activity (i.e., persistent inflammation) on [^{18}F]FDG-PET/CT images in lung tissue of normal appearance and in mediastinal lymph nodes. To our best knowledge, [^{18}F]FDG-PET/CT has not previously been used to detect residual inflammatory processes after COVID-19 infection. These findings contribute evidence on the pathophysiological processes in patients who survive hospital admission for COVID-19 pneumonia.

The [^{18}F]FDG-PET/CT has been employed in patients with influenza A, aspiration pneumonia, and organized pneumonia to assess the extent and severity of the disease, to follow its course, and to evaluate the response to therapy [5,18,19]. Research on the role of [^{18}F]FDG-PET/CT in COVID-19 infection has generally focused on the acute phase. In this regard, Qin et al. reported high [^{18}F]FDG uptake in lung lesions and mediastinal lymph nodes of four patients strongly suspected of the infection [4], and Colandrea et al. described elevated [^{18}F]FDG uptake in lung lesions in 80% of a series of symptom-free oncology patients diagnosed with COVID-19 [20]. However, few studies have addressed the short- or medium-term consequences of COVID-19 infection. Dietz et al. recently reported increased [^{18}F]FDG uptake in lung lesions and mediastinal lymph nodes of 13 non-critically ill COVID-19 patients at days 6–14 after symptom onset, although the short-axis diameter of mediastinal lymph nodes was always < 1 cm [3]. Johnson et al. proposed that high [^{18}F]FDG uptake in mediastinal lymph nodes might be secondary to lung involvement in COVID-19 [21]. Bai et al. found elevated metabolic activity in residual lung lesions in COVID-19 survivors after two successive negative results in the RT-PCR test [22], and Scarlattei et al. reported that this metabolic activity remained high many weeks after the disappearance of symptoms and a negative RT-PCR test result [23]. The present results are in line with the above findings and contribute novel data on increased metabolic activity in lung tissue of normal appearance and in mediastinal lymph nodes of normal size. In this context, Xu et al. described lymphocyte-dominated interstitial mononuclear inflammatory infiltrates in both lungs of a patient with COVID-19 and reported that substantial inflammation may persist in the lungs after the disappearance of the infection [24]. The elevated [^{18}F]FDG uptake would reflect increased glycolytic activity due to infiltration and inflammation of the lung, even in normally aerated areas that show no morphological alterations on CT images, demonstrating the greater capacity of [^{18}F]FDG-PET/CT to detect inflamed lung areas in comparison to CT alone [8,22], which

may persist long after the disappearance of COVID-19 infection. The possible duration of the post-COVID-19 inflammatory response in lungs and extrapulmonary sites has yet to be established and warrants further research.

At 2–3 months post-discharge, patients with elevated chest [^{18}F]FDG uptake were older and characterized by a higher Charlson index, more frequent fatigue and respiratory distress, and lower hemoglobin and lymphocyte counts in comparison to those with normal [^{18}F]FDG uptake. The SUVpeak of the target lesion and pulmonary TLG were significantly correlated with acute phase reactants and white blood cell counts at admission, during the hospital stay, and at 2–3 months post-discharge. Although there is a lack of similar studies in severely ill COVID-19 survivors for comparison with these results, they are consistent with previous findings on risk factors for more severe infection, including old age, underlying comorbidities [12,25], and similar changes in white blood cell counts, lymphocyte counts, procalcitonin and CRP levels, and NLR [26,27]. The [^{18}F]FDG-PET/CT findings were correlated with the NLR in all studied phases of COVID-19 disease. The persistence over time of increased [^{18}F]FDG uptake intensity may reflect a more severe acute phase of the disease.

The lung appears to be the most frequently involved organ in COVID-19, with reports of diffuse alveolar epithelium destruction, capillary damage/bleeding, hyaline membrane formation, alveolar septal fibrous proliferation, and/or pulmonary consolidation, among others [12,24]. Long-term follow-up studies of survivors of other coronavirus infections found that respiratory function limitations frequently last for months or even years, including impaired DLCO (in 15.5–43.6% of patients) and decreased TLC (5.2–10.9%) [28–30]. Various authors have addressed short- and medium-term respiratory function outcomes in survivors of COVID-19 infection, usually at hospital discharge [31,32]. In a study at 2–3 months post-discharge of 55 COVID-19 survivors who had not required mechanical ventilation, Zhao et al. described residual pulmonary function in 14 patients (25.45%), mainly impaired DLCO (in 13.6%) [33]. In a study at 6 weeks post-discharge of 124 COVID-19 survivors, van den Borst et al. [34] described an improvement in radiological images for almost all patients (99%) but observed residual lung parenchymal alterations in 91% of the patients and reduced lung diffusion capacity in 42%. Likewise, in their study at 3 months post-discharge of 76 healthcare workers who recovered from COVID-19, Liang et al. reported normal FEV1, FVC, FEV1/FVC, TLC, and DLCO values (>80% predicted) in 82% of the patients but the persistence of mild pulmonary function abnormalities in 42% [35]. The proportion of the present patients with impaired pulmonary function at 2–3 months was in line with previous findings on the short- to medium-term effects of COVID-19 infection [33,34].

The most frequent respiratory sequela of COVID-19 was DLCO alteration, as reported in previous studies, which may indicate the presence of pulmonary fibrosis [12,24]. DLCO and other respiratory function parameters were negatively correlated with the lung [^{18}F]FDG uptake as quantified by TLG. Although only a small proportion of the present patients had severe airway dysfunction, the results suggest that COVID-19 produces diffuse pulmonary epithelial damage and mild congestion of the airway mediated by lymphocyte-dominated interstitial inflammatory infiltrates. No published data appear to be available on the association between respiratory function test results and pulmonary TLG. The majority of the present patients showed no lung lesions on CT scans at 2–3 months after discharge; however, pulmonary function was impaired in more than half of the patients with a normal lung CT scan. Hence, pulmonary function and [^{18}F]FDG-PET/CT testing is more sensitive than CT alone for identifying candidates for pulmonary rehabilitation after SARS-CoV-2 pneumonia [22].

High [^{18}F]FDG uptake may be related to increased anaerobic glycolysis caused by a cascade of reactions involving inflammatory cells [7,36]. In this way, the uptake of [^{18}F]FDG by lung lesions and lymph nodes observed in this study may be due to nonspecific immune or inflammatory activation, similar to the high [^{18}F]FDG uptake observed in lung lesions

caused by the Middle East respiratory syndrome, pandemic H1N1 influenza virus, and organized pneumonia [18,19,37].

The [^{18}F]FDG-PET/CT offers a complementary approach to other imaging modalities by providing metabolic information. Although not currently recommended for the diagnosis of COVID-19 in the acute phase [8], it can yield relevant information for the diagnosis of short- and medium-term complications, including the chronic damage to the lungs and extrapulmonary sites that can follow acute infection [6,22]. However, radiologists and nuclear physicians need to develop a thorough understanding of the cellular mechanisms that underlie the pathophysiology of COVID-19 in the clinical settings of lung and extrapulmonary malignancies and inflammatory diseases in order to avoid misinterpretation of [^{18}F]FDG-PET/CT images [31].

Besides the small sample size, the main limitation of this study was the absence of a control group, hampering the possibility to detect causal relationships between the findings and COVID-19 infection. The epidemiological environment in which this study was carried out determined strict, restrictive conditions for access to hospital centers in our center and population. Evidently, the performance of [^{18}F]PET/CT in healthy collaborating patients was obviously not authorized. In addition, no test results were available for the baseline respiratory function of patients before COVID-19, although the presence of chronic lung disease was an exclusion criterion. Further research is required to fully elucidate the impact of COVID-19 on pulmonary function. In this regard, the present results cannot be extrapolated to patients with chronic lung disease. Another study limitation was the absence of a follow-up period to explore the long-term clinical relevance of the respiratory function impairment. Finally, biopsy specimens were not available for the studied organs. Nevertheless, the present findings contribute to laying the foundations for future studies with larger series on the potential role of [^{18}F]FDG-PET/CT in evaluating the sequelae of COVID-19 infection. These should have prolonged follow-up periods to explore the possible relationship between initial lung inflammation and long-term sequelae such as residual lung fibrosis and respiratory failure.

5. Conclusions

In conclusion, at 2–3 months after the acute phase of SARS-CoV-2 infection, almost half of the patients evidenced an impairment of pulmonary function that was correlated with [^{18}F]FDG-PET/CT findings. In addition, the increased metabolic activity observed in the lung and mediastinal lymph node was associated with clinical and laboratory markers of disease severity. The [^{18}F]FDG-PET/CT is useful to obtain novel information on the pathogenesis of COVID-19 and on the diagnostic and evaluation of short- and medium-term sequelae, contributing to their management.

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Conflicts of Interest: All authors declare that they do not have conflicts of financial or non-financial interest. Written consent was obtained for all patients and a local ethics committee approved the study.

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11.3 Artículo 3.

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Abnormal Alpha-1 Antitrypsin Levels and Other Risk Factors Associated with Lung Function Impairment at 6 and 12 Months after Hospitalization Due to COVID-19: A Cohort Study

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Abstract: Respiratory function deficits are common sequelae for COVID-19. In this study, we aimed to identify the medical conditions that may influence lung function impairment at 12 months after SARS-CoV2 infection and to analyze the role of alpha-1 antytripsin (AAT) deficiciency (AATD). A cohort study was conducted on hospitalized COVID-19 pneumonia patients in Granada (Spain) during the first infection wave who were referred to a post-COVID-19 hospital clinic. The patients were monitored with three follow-up visits from May 2020 to May 2021. Previous medical history, hospital admission data, baseline parameters and physical examination data were collected at the first visit. Pulmonary function tests were performed at 6 and 12 months together with the determination of AAT level and AATD genotype. After 12 months, 49 out of 157 patients (31.2%) continued to have lung function impairment. A multivariate analysis showed a statistically significant association of lung function impairment with: higher Charlson index; pneumonia with a central and/or mixed distribution; anemia on admission; time in intensive care; need for corticosteroid boluses; abnormal respiratory sounds at 6 months; elevated lactate dehydrogenase at 12 months; abnormal AAT; and MZ genotype. Our results suggest that these medical conditions predispose COVID-19 patients to develop long-term lung function sequelae.

Keywords: COVID-19; respiratory function tests; alpha-1 antitrypsin; long-term follow-up

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1. Introduction

The term "Post-COVID-19 conditions" defined by the World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) is currently used to refer to continuous or new persistent symptoms, secondary sequelae from organ damage and effects of the disease or hospitalization that appear after the acute infection caused by Severe Acute Respiratory Syndrome (SARS) coronavirus 2 (SARS-CoV-2) [1,2]. Since other systemic viral diseases [3] and epidemics caused by previous coronaviruses (e.g., SARS-CoV-1, Middle East Respiratory Syndrome coronavirus [MERS]) have been associated with post-infectious sequelae and long-term complications [4,5], it was expected that they would also develop in post-COVID-19 patients. In this sense, alterations in respiratory function secondary to COVID-19 infection have been described, but they are not always related to the severity of the disease [6,7].

The CDC proposed α1-antitrypsin (AAT) deficiency (AATD) as a possible medical condition deserving further study due to mixed levels of evidence [8]. AAT is a

glycoprotein belonging to the serpin group whose main function is to inhibit neutrophil elastase and prevent excessive proteolytic degradation of the connective tissue of the lungs [9,10]. AATD is a rare genetic disease due to mutations of the *SERPINA1* gene that produce low levels or defective AAT in the blood. This increases the risk of developing a variety of diseases, including pulmonary emphysema. Recent studies have described how this protein has biological functions that can counteract both SARS-CoV-2 infection and the underlying pathophysiological processes. It has also been suggested that patients with genotypic alterations have a higher risk of severity and even death [11,12]. In addition, a 2004 analysis of previous epidemics with other coronaviruses showed that serum samples from SARS patients had dramatically elevated levels of truncated forms of AAT, which correlated with the severity of SARS and indicated that these truncated forms of AAT could serve as SARS biomarkers with 100% sensitivity [13].

With the above in mind, the primary objective of the present study was to identify which medical conditions may influence the development of impaired lung function 12 month after COVID-19 infection. The secondary objective was to analyze the role of AATD and its related genetic mutations in such functional alterations.

2. Material and Methods

2.1. Study Design

This was a prospective, cohort study that included patients admitted to hospital for COVID-19 pneumonia from February to May 2020 (i.e., non-vaccinated patients from the "first wave" of COVID-19 in Spain) who were referred for follow-up to a post-COVID-19 respiratory clinic at the Virgen de las Nieves University Hospital in Granada, Spain. The patients were followed from May 2020 to May 2021. All were enrolled consecutively, and three follow-up visits were scheduled (Figure 1).

For COVID-19 diagnosis, RT-PCR from upper respiratory tract samples (nasopharyngeal or oropharyngeal swab) or lower respiratory tract samples (sputum collection) with antibody serology (IgM and IgG) by ELISA were used. The patients were followed for one year after the acute infection.

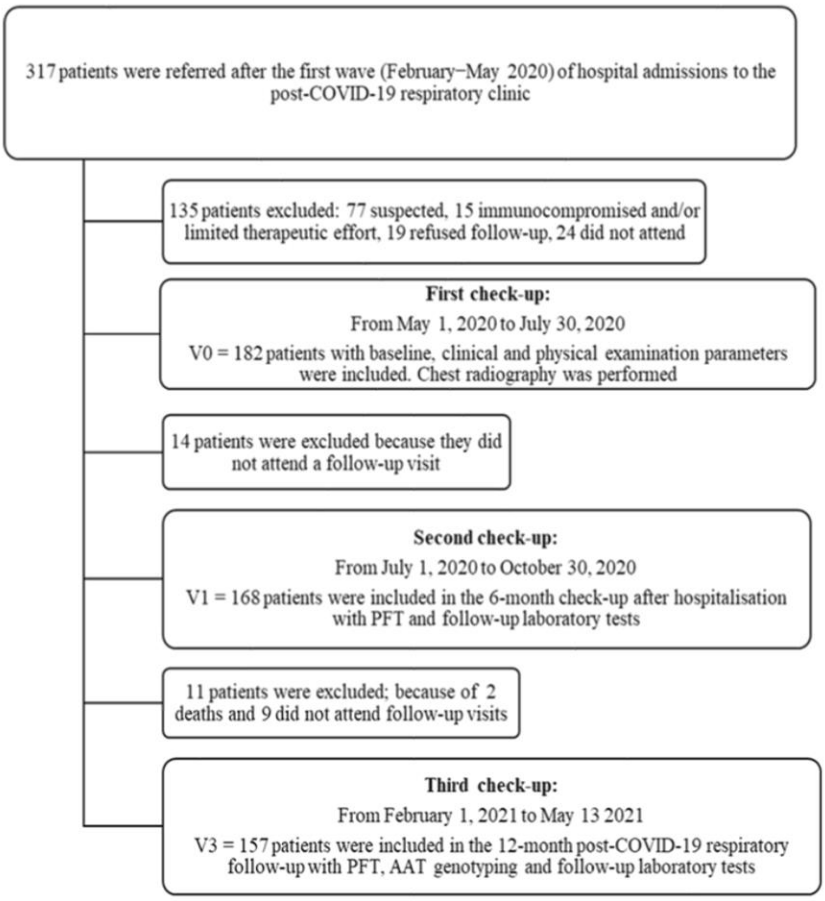

Figure 1. Patient flow chart after acute COVID-19 respiratory infection with first, second and third follow-up visits carried out at the University Hospital Virgen de las Nieves.

The first follow-up visit was two months after discharge from hospital. At that visit, previous medical history, characteristics of the hospital admission, baseline parameters and physical data were collected, and an X-ray was performed. The second follow-up was six months after discharge. Pulmonary function tests (PFT) and a 6-min walk test (6MWT) were performed. The last follow-up was carried out 12 months after hospital discharge and included laboratory tests and AAT levels along with genotyping and repeat PFT for those who had any abnormality in previous tests.

The study was carried out in accordance with the requirements stipulated in the Declaration of Helsinki (2013 revision) and Spanish Organic Law 15/1999 on the Protection of Personal Data. The study was approved by the Hospital's Ethics Committee, assigning it the internal code 1017-N-20.

2.2. Study Population

The inclusion criteria were: patients over 18 years of age with a confirmed diagnosis of SARS-CoV-2 infection (according to international recommendations [14]), hospitalization for COVID-19 pneumonia and informed consent. Exclusion criteria were: patients without confirmation of SARS-CoV2 infection, patients with underlying immunosuppression and patients in whom therapeutic efforts were limited.

2.3. Study Variables

2.3.1. During hospitalization

Data were collected on demographics, medical history and characteristics of the hospitalization, especially the laboratory parameters related to the severity or mortality of the disease [15] (Table S1 in the supplementary material).

During the patients' hospital stay, chest X-rays in posterior–anterior and lateral projections were performed at diagnosis. Results were reported according to the recommendations of the Sociedad Española de Radiología Médica (SERAM) [Spanish Society of Medical Radiology], the international standard nomenclature defined by the Fleischner Society glossary, and available publications at the time of reporting [16,17]. The features of each X-ray performed were described in terms of density (alveolar, ground glass or mixed), distribution (central, peripheral or diffuse), location (unilateral or bilateral) and extension (unilobar or multilobar).

2.3.2. During follow-up

At the 6-month follow-up, PFT with spirometric measurements, lung volumes and carbon monoxide diffusing capacity and a 6-min walk test (6MWT) were scheduled. The functional examination was carried out by experienced personnel using Jaeger MasterScreen Body whole body plethysmograph equipment (CareFusion Germany 234 GmbH, Hoechberg, Germany). Reference values and acceptability criteria were based on European and Spanish standards [18,19].

At the 12-month follow-up visit, further PFT were performed only on patients with functional alterations at the previous check-up. Plasma concentrations of AAT and C-reactive protein (CRP) were determined in all patients to rule out temporary elevation of AAT levels due to inflammation [20]. Genotyping of AAT was performed from a mouth swab using the Progenika A1AT Genotyping Test (Progenika Biopharma, a Grifols Company, Derio, Spain). The test allows simultaneous analysis of up to 384 samples per batch and is able to identify the 14 most frequent deficiency variants of the *SERPINA1* gene; details are available elsewhere [21].

The Progenika clinical service laboratory sequenced the *SERPINA1* gene if they did not find any of the 14 mutations and the serum AAT level was <60 mg/dL, or at the request of the treating physician. Sequencing of the seven exons of the gene was performed using latest generation-NGS techniques (MiSeq, Illumina Inc., San Diego, CA, USA); details are available elsewhere [21].

2.4. Statistical Analysis

In the descriptive analyses, continuous variables are given as mean and standard deviation. Categorical variables are given as numbers and percentages. For the comparison of quantitative data between the two groups for PFT variables altered at 12 months and abnormal AAT values, Student's t test for parametric independent data and the Mann–Whitney U test for non-parametric data were used. For quantitative data comparisons between qualitative variables with three or more groups, ANOVA was applied with the Bonferroni correction for multiple comparisons in the case of normality and the Kruskal–Wallis test otherwise. The association between the categorical variables was assessed by means of contingency tables applying the chi-square test for individual comparisons or Fisher's exact test for multiple comparisons. Univariate binary logistic regression analysis

was used to study the association between the different variables related to baseline comorbidities, demographic, clinical and physical characteristics and laboratory tests and functional respiratory recovery at 12 months after hospital discharge. Finally, a multivariate model was adjusted from those variables that showed a significant association in the univariate analysis. Data were processed for analysis using IBM SPSS Version 25.0 (IBM Corp, Armonk, New York, NY, USA) and/or mathematical computing R software. A p value < 0.05 was considered statistically significant.

3. Results

Out of 317 patients screened, a total of 182 patients hospitalised for SARS-CoV-2 infection were referred to post-COVID-19 respiratory clinics for review. The follow-up study lasted for one year after the acute infection with 168 patients (92.3%) attending the 6-month check-up and 157 (86.3%) the 12-month check-up (Figure 1).

The clinical characteristics of the study population separated according to normal or impaired lung function at 12 months are shown in Table 1. Details of laboratory parameters are provided in Table S2 of the supplementary material.

Table 1. Baseline comorbidities, demographics, clinical and physical characteristics of the patients at admission, during hospitalization and at the 12-month follow-up visit.

Baseline Characteristics	Lung Function at 12 Months [Mean ± SD or n (%)]		*p*-Value
	Normal (n = 108)	Impaired (n = 49)	
Age	57.4 ± 12.5	62.4 ± 13.0	0.026
Male	68 (63)	25 (51)	0.272
Smoking history:			
Active smoker	6 (5.6)	3 (6.1)	0.201
Never smoked	64 (59.3)	25 (51)	
Former smoker	38 (35.2)	21 (35.6)	
Cumulative smoking burden index (smokers and former smokers)	11.3 ± 19.9	14.8 ± 24.9	0.039
BMI (kg/m²)	29.4 ± 5.0	29.1 ± 5.0	0.938
Previous medical history			
≥2 baseline comorbidities	37 (34.3)	31 (63.3)	0.006
HBP	45 (41.7)	25 (51)	0.413
DM2	10 (9.3)	13 (26.5)	0.006
Dyslipidemia	23 (21.5)	14 (28.6)	0.599
Coronary heart disease	3 (2.8)	1 (2)	0.075
Cardiac arrhythmias	4 (3.7)	4 (8.2)	0.715
Cerebrovascular disease	2 (1.9)	3 (6.1)	0.391
Baseline respiratory comorbidities	22 (20.4)	20 (40.8)	0.008
COPD	3 (2.8)	8 (16.3)	0.006
Baseline liver disease	3 (2.8)	2 (4.1)	0.638
Chronic kidney disease	6 (5.6)	4 (8.2)	0.633
Active cancer	1 (2%)	1 (0.6%)	0.401
Previous cancer history < 5 years	5 (4.7)	5 (10.2)	0.526
Charlson index ≥ 3	32 (29.6)	32 (65.3)	0.002
Signs and symptoms on admission			
Fever (>37.5 °C)	103 (72.5)	39 (79.6)	0.002
Dyspnoea	62 (57.9)	39 (79.6)	0.010
Cough	79 (73.8)	38 (77.6)	0.638
Exhaustion	83 (77.6)	31 (63.3)	0.064

Musculoskeletal pain	60 (56.1)	17 (35.4)	0.019
Anosmia/ageusia	24 (22.4)	13 (26.5)	0.449
Gastrointestinal symptoms	27 (64.3)	15 (30.6)	0.441
Cardiac symptoms	4 (3.7)	3 (6.1)	0.441
Neurological symptoms	16 (15)	8 (16.3)	0.754
Dermatological symptoms	1 (0.9)	1 (2.0)	0.270
ARDS (PaO2/FiO2 < 300 mmHg)	21 (19.6)	19 (38.8)	0.012
Hospitalization			
Mean length of stay (days)	9.9 ± 11.2	14.8 ± 14.0	0.043
Pneumonia on admission	101 (93.5)	41 (83.7)	0.047
Characteristics of pneumonia on chest X-ray (n = 142)			
-Density:			
• Alveolar	25 (24.8)	13 (31.7)	0.247
• Ground-glass opacity (GGO)	19 (18.8)	5 (12.2)	
• Mixed	57 (56.4)	23 (56.1)	
-Distribution:			
• Central	6 (5.9)	8 (19.5)	0.047
• Peripheral	68 (67.3)	17 (41.5)	
• Mixed	27 (26.7)	16 (39)	
-Location:			
• Unilateral	10 (9.9)	5 (12.2)	0.505
• Bilateral	91 (90.1)	36 (87.8)	
-Involvement:			
• Single lobe	9 (8.9)	3 (7.3)	0.827
• Multilobar	92 (91.1)	38 (92.7)	
Admission to the ICU	3 (2.8)	6 (12.2)	0.030
Endotracheal intubation (ETI)	2 (1.9)	6 (12.2)	0.017
Treatment received:			
• Corticosteroids (prednisone, methyprednisolone)	39 (58.2)	28 (41.8)	0.019
• Antivirals	92 (68.1)	43 (31.9)	0.625
• Tocilizumab	7 (43.8)	9 (56.3)	0.031
• Anakinra	3 (75)	1 (25)	0.679
• Antimalarials (hydroxychloroquine)	102 (69.4)	45 (30.6)	0.182

The results are expressed as frequency (%) and mean (±SD). Abbreviations: BMI = body mass index, ARDS = acute respiratory distress syndrome, IL-6 = interleukin 6; COPD = chronic obstructive pulmonary disease; DM2 = diabetes mellitus-2; HBP = high blood pressure; ICU = intensive care unit; ETI = endotracheal intubation.

The study population was predominantly males (93 [59.2%]) with a mean age of 59.9 years (±12.9) and a mean BMI of 29.4 (±5.0). Ninety-eight patients (62.4%) were active smokers or former smokers with a mean cumulative smoking burden index (ICAT) of 12.4 (±21.6) pack-years. A total of 68 patients (43.3%) had two or more baseline comorbidities with a Charlson index of 3 or higher in 64 patients (40.8%). The mean hospital stay was 11.5 days (±2.8), and the most common clinical manifestations while in hospital (affecting over 25% of patients) were fever, dyspnoea, cough, fatigue, musculoskeletal pain and gastrointestinal symptoms (Table 1). The admission chest X-ray showed pneumonia in 142 cases (90.4%). Sixty-seven patients (43.2%) received corticosteroids (prednisone and/or methylprednisolone) and 135 (87.1%) received antiretrovirals during treatment.

3.1. Association between the Patients Clinical Characteristics and Lung Function 12 Months after Hospital Discharge

Of the total of 157 patients who completed the study, 49 (31.2%) met the criteria for abnormal PFT at 12 months, i.e., they had impaired lung function. The remaining 108 patients (68.8%) had PFT within normal limits.

The comparison of the two groups (abnormal vs. normal PFT) using univariate binary logistic regression analysis is shown in Table 2.

Table 2. Risk factors associated with impaired lung function at 12 months for the bivariate and multivariate analysis.

Covariate	OR [a]	*p*-Value	95% CI for OR	Explained Variance, R^2
(A) Univariate logistic regression analysis				
Age	1.032	0.026	1.004–1.061	0.046
Charlson index	1.421	<0.001	1.186–1.703	0.139
≥2 comorbidities	3.305	0.001	1.635–6.680	0.100
DM2	3.539	0.006	1.426–8.779	0.065
Heart disease	2.833	0.075	0.899–8.931	0.028
Lung disease	2.696	0.008	1.290–5.636	0.061
COPD	6.829	0.006	1.727–27.013	0.075
Signs and symptoms on admission				
Fever	0.151	0.002	0.045–0.511	0.091
Dyspnoea	2.831	0.010	1.280–6.260	0.064
Asthenia	0.498	0.064	0.238–1.041	0.030
Myalgia	0.430	0.019	0.212–0.869	0.051
X-ray distribution				
Peripheral	Ref			0.095
Central	5.333	0.006	1.632–17.434	
Mixed	2.370	0.038	1.049–5.357	
Haemoglobin (g/dL)	0.780	0.017	0.636–0.956	0.054
Leucocytes (count ×10^3/μL)	1.172	0.011	1.037–1.324	0.061
Neutrophils (count ×10^3/μL)	1.175	0.024	1.022–1.352	0.051
Lymphocytes (%)	0.957	0.044	0.916–0.999	0.041
NLR	1.144	0.006	1.040–1.259	0.080
Platelets (count ×10^3/μL)	1.004	0.012	1.001–1.008	0.061
Hospitalization				
ICU admission	4.884	0.030	1.168–20.420	0.045
ETI	7.395	0.017	1.436–38.089	0.060
ARDS	2.594	0.012	1.229–5.474	0.055
Corticosteroid therapy	2.291	0.019	1.149–4.566	0.050
Corticosteroid boluses	3.665	<0.001	1.765–7.612	0.107
Tocilizumab	3.182	0.031	1.109–9.128	0.041
Haemoglobin (g/dL)	0.759	0.008	0.620–0.930	0.076
Albumin (g/dL)	0.439	0.048	0.195–0.991	0.043
LDH (U/L)	1.004	0.011	1.001–1.007	0.065
Procalcitonin	10.160	0.052	0.982–105.105	0.062
IL-6 (pg/mL)	1.107	0.090	0.997–1.036	0.120
LTOT	4.921	0.003	1.701–14.235	0.080
Length of hospital stay (days)	1.003	0.043	1.001–1.066	0.045
Follow-up				
Haemoglobin (g/dL)	0.763	0.012	0.619–0.941	0.061

Platelets (count ×10³/μL)	1.005	0.028	1.001–1.010	0.051
LDH (U/L)	1.011	0.017	1.002–1.021	0.063
Ferritin (ng/mL)	0.995	0.023	0.991–0.999	0.065
Troponin (ng/L)	0.782	0.007	0.654–0.934	0.100
Vitamin D (ng/mL)	0.955	0.058	0.910–1.002	0.042
Abnormal respiratory sounds	7.186	0.018	1.395–37.023	0.059
(B) Multivariate logistic regression analysis				
Charlson index	1.336	0.030	1.029–1.735	
X-ray distribution				
Peripheral	Ref			
Central	10.820	0.004	2.093–55.934	
Mixed	4.855	0.014	1.374–17.154	
Admission haemoglobin (g/dL)	0.604	0.006	0.422–0.864	0.534
ICU admission	33.184	0.012	2.180–505.072	
Methylprednisolone boluses	3.447	0.043	1.039–11.433	
Follow-up LDH (U/L)	1.025	0.004	1.008–1.042	
Abnormal respiratory sounds	15.157	0.027	1.011–227.244	

OR = odds ratio; Ref = reference category; LDH = lactate dehydrogenase; NRL = neutrophil/lymphocyte ratio; IL-6 = interleukin 6; LTOT = long-term oxygen therapy. ICU = intensive care unit; ETI = endotracheal intubation; ARDS = acute respiratory distress syndrome; DM2 = Diabetes mellitus type-2. (a) The OR account for a 1 unit increase in each of the independent variables.

After univariate analysis of the association of the different variables with the risk of having impaired lung function, a multivariate model was adjusted, which initially contained all the variables. From this model, a predictive model was adjusted that included the following as predictors of alterations of lung function 12 months after hospitalization: the Charlson index; pneumonia with a mixed and/or central distribution; anaemia on admission; admission to the ICU; need for treatment with corticosteroid boluses; persistence of abnormal respiratory sounds at the 6-month check-up; and lactate dehydrogenase (LDH) elevation at the 12-month follow-up (Table 2).

3.2. Lung Function at 6 and 12 Months after Hospitalization

All patients who attended the 6-month follow-up were asked to undergo PFT and a 6MWT, but only 150 completed these tests. Of that 150, 67 (42.7%) had some type of functional impairment. The same patients were asked to undergo repeat PFT at the 12-month check-up, and 49 patients (31.2%) continued to have impaired lung function. Therefore, 18 patients (11.5%) with abnormal PFT at 6 months had recovered functionally at 12 months (Figure 2). The serial values for the PFT at 6 and 12 months post discharge in the patients who had pulmonary functional alterations are shown in Table 3.

Table 3. Serial values of pulmonary function tests at 6 and 12 months in the subgroup of patients with impaired lung function. Data are expressed as percentage (%) or mean ± standard deviation (SD).

Respiratory Function Parameters	At 6 Month (n = 67) (Mean ± SD)	At 12 Month (n = 49) (Mean ± SD)	Difference (Mean ± SD)	95% CI	*p*-Value
FVC (%)	91.2 ± 17.5	92.2 ± 19.7	−0.98 ± 12.4	−4.340–2.369	0.558
FEV1 (%)	87.1 ± 20.3	90.8 ± 21.6	−3.6 ± 16.0	−7.958–0.708	0.099
FEV1/FVC	75.0 ± 11.5	77.4 ± 10.2	−2.4 ± 9.4	−4.909–0.176	0.067
TLC (%)	98.0 ± 18.4	102.6 ± 21.9	−4.6 ± 14.6	−8.671–0.555	0.027
DLCO (%)	73.6 ± 19.0	76.6 ± 13.5	−2.9 ± 17.6	−7.925–2.069	0.245

KCO (%)	92.3 ± 20.8	95.1 ± 16.1	−2.8 ± 17.9	−7.866–2.282	0.274
RV (%)	100.4 ± 30.8	105.6 ± 47.0	−5.3 ± 38.4	−15.96–5.41	0.327
Distance 6MWT (m)	466.4 ± 107.9	430.0 ± 114.7	36.4 ± 79.9	12.95–59.89	0.003
Initial SaO_2 6MWT %)	96.5 ± 1.8	95.8 ± 2.4	0.71 ± 2.6	−0.040–1.456	0.063
Final SaO_2 6MWT %)	93.7 ± 4.0	92.7 ± 4.2	1.0 ± 3.3	0.000–1.917	0.05
Initial—final SaO_2 6MWT (%)	2.9 ± 4.2	3.2 ± 4.3	−0.25 ± 4.1	−1.452–0.952	0.677

FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; TLC = total lung capacity; RV = residual volume; DLCO = diffusing capacity for carbon monoxide; 6MWT = six minute walk test; SaO_2 = oxygen saturation.

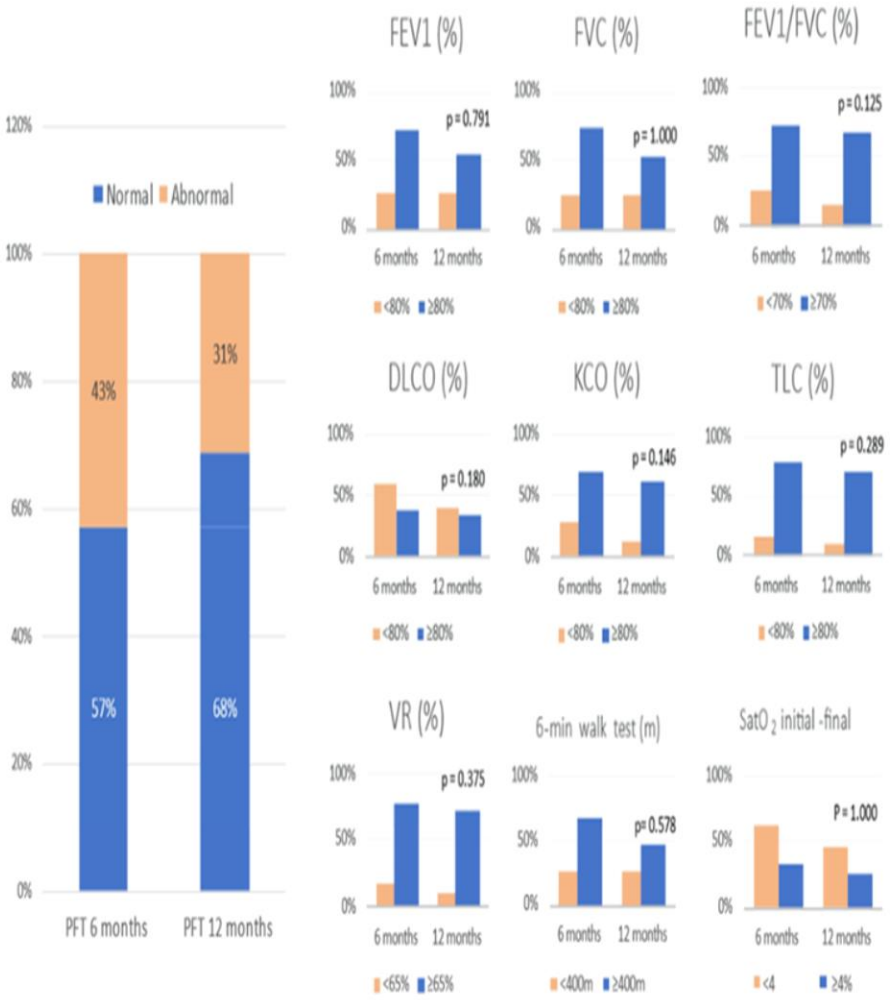

Figure 2. Evolution of pulmonary function tests at 6 and 12 months follow-up. Data are expressed as percentege (%). Abbreviations: FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; TLC = total lung capacity; RV = residual volume; DLCO = carbon monoxide transfer by single breath; KCO= diffusion constant for carbon monoxide; VR = residual volume; 6MWT = six-minute walking test; $SatO_2$ = oxygen saturation.

3.3. Association between AAT Levels and Genotyping and Functional Recovery

The data from the 157 patients who attended the third follow-up were categorised according to genotype with the corresponding plasma levels of AAT and elevated CRP value > 5 mg/dL (Table S3, supplementary material); 83.4% of hospitalised patients had normal AAT genotype (Pi*MM), with plasma AAT levels of 128.6 ± 1.4 mg/dL. Other genotypic variants found were Pi*MS (14.6%), Pi*MZ (1.3%) and Pi*M/P Lowell (0.6%) with plasma AAT levels of 116.4 ± 3.2, 89.0 ± 15.0 and 114.8 ± 0.0 mg/dL, respectively.

Figure 3 shows a graph of the mean plasma levels of AAT for each category of AAT genotype analysed with patients stratified based on the CRP level (categorised as normal if ≤5 mg/L or elevated if >5 mg/L) for all hospitalised patients and for the subgroups with normal or impaired lung function at 12 months.

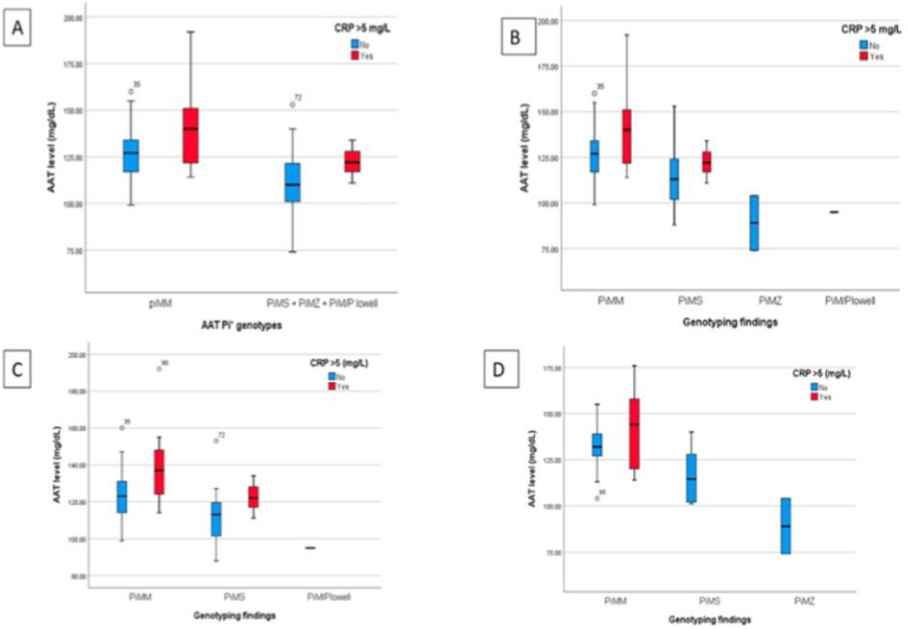

Figure 3. Findings from the plasma determination of AAT, allelic genotype and CRP determination for all hospitalised patients and for the subgroups of patients with normal or impaired lung function at 12 months. (**A**) Breakdown of AAT levels classified by normal genotype (Pi*MM) or unified heterozygous deficiencies (Pi*MS, Pi*MZ and Pi*M/P Lowell) and stratified by normal or elevated CRP for all hospitalised patients. (**B**) Comparison of AAT levels based on genotyping (Pi*MM, Pi*MS, Pi*MZ and Pi*M/P Lowell) and the normal or high CRP value for hospitalised patients. C and D) AAT values analyzed based on genotype and normal or elevated CRP of the subgroups of patients with normal (**C**) or impaired lung function (**D**) at the 12 month follow-up.

Table 4 shows how normal AAT plasma levels (range 83–220 mg/dL) or abnormal levels, along with the allelic genotype detected (Pi*MM, Pi*MS, Pi*MZ and Pi*M/P Lowell), influence the recovery of respiratory function variables at 6 and 12 months of follow-up.

Table 4. Association between alpha-1 antitrypsin (AAT) levels results and allelic genotype with pulmonary function test parameters at 6 and 12 month follow-up visits.

	AAT Levels (mg/dL)			Genotype				
	Normal	Abnormal	*p*-Value	Pi*MM	Pi*MS	Pi*MZ	Pi*MP Lowell	*p*-Value
(A) Abnormal PFT (mean ± SD) at 6 months (N = 66)								
FEV1 %	99.9 ± 17.8	69.9 ± 22.8	0.020	100.4 ± 18.7	92.1 ± 17.2	78.9 ± 35.4	114.8	0.086
FVC %	106.7 ± 76.0	79.7 ± 10.9	0.618	101.0 ± 16.4	134.0 ± 183.9	86.3 ± 20.2	113.7	0.244
FEV1/FVC %	78.4 ± 7.0	66.4 ± 13.6	0.018	78.0 ± 8.1	77.0 ± 9.1	69.8 ± 18.5	79.1	0.549
TLC %	102.9 ± 14.8	79.5 ± 19.1	0.029	103.5 ± 14.7	102.6 ± 16.3	74.9 ± 12.5	107.8	0.065
DLCO %	88.6 ± 22.1	80.4 ± 40.7	0.607	89.5 ± 22.2	85.2 ± 19.8	47.7 ± 5.6	103.4	0.045
KCO %	100.2 ± 17.7	110.0 ± 25.5	0.438	99.9 ± 18.0	100.1 ± 17.4	79.4 ± 17.8	109.4	0.414
RV %	102.1 ± 26.4	76.8 ± 22.9	0.181	100.4 ± 24.8	114.2 ± 36.1	69.5 ± 12.6	93.1	0.055
6MWT Distance (m)	507.0 ± 110.3	568.5 ± 115.3	0.436	516.0 ± 100.8	494.5 ± 115.1	306.0 ± 256.0	-	0.040
6MWT Initial SaO_2 (%)	96.5 ± 1.5	95.5 ± 2.1	0.346	96.6 ± 1.6	96.6 ± 1.5	95.0 ± 2.8	-	0.567
6MWT Final SaO_2 (%)	95.0 ± 3.8	91.5 ± 5.0	0.198	95.0 ± 3.9	95.0 ± 3.9	89.5 ± 2.1	-	0.224
(B) Abnormal PFT (mean ± SD) at 12 months (N = 49)								
FEV1 %	95.9 ± 20.4	69.4 ± 23.6	0.075	97.0 ± 19.7	94.1 ± 24.1	64.1 ± 16.6	-	0.083
FVC %	77.7 ± 9.2	64.9 ± 14.4	0.257	98.3 ± 19.5	95.6 ± 19.8	71.1 ± 2.8	-	0.149
FEV1/FVC %	96.9 ± 2.3	81.3 ± 8.2	0.059	77.6 ± 8.9	76.7 ± 13.0	70.8 ± 22.8	-	0.616
TLC %, SD	104.9 ± 20.1	75.0 ± 12.2	0.041	105.7 ± 20.5	100.6 ± 16.7	74.9 ± 12.2	-	0.087
DLCO %, SD	82.7 ± 15.1	75.5 ± 40.2	0.525	82.8 ± 15.5	81.1 ± 11.0	47.0	-	0.067
KCO %, SD	111.9 ± 118.7	101.6 ± 23.5	0.903	111.3 ± 117.1	98.0 ± 15.5	84.9	-	0.916
RV %, SD	108.7 ± 41.8	59.9 ± 5.9	0.105	109.8 ± 42.3	92.2 ± 4	66.5 ± 15.3	-	0.196
6MWT Distance (m)	442.8 ± 104.2	567.5 ± 24.7	0.098	443.3 ± 102.0	441.9 ± 107.0	550.0	-	0.588
6MWT Initial SaO_2 (%)	95.7 ± 2.2	97.5 ± 2.1	0.258	95.7 ± 2.3	95.5 ± 1.9	99.0	-	0.329
6MWT Final SaO_2 (%)	93.3 ± 3.9	94.5 ± 2.1	0.662	92.9 ± 4.0	94.6 ± 2.7	96.0	-	0.317

Abbreviations: SD = standard deviation FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; TLC = total lung capacity; RV = residual volume; DLCO = diffusing capacity for carbon monoxide transfer in single breath; 6MWT = six minute walk test; SaO_2 = oxygen saturation; AAT= Alpha-1 antitrypsin; PFT= pulmonary function test.

At 6 months, the functional parameters of forced expiratory volume in the first second (FEV1) (69.9 ± 22.8 vs. 99.9 ± 17.8; $p = 0.02$), FEV1/forced vital capacity (FVC) (66.4 ± 13.6 vs. 78.4 ± 7.0, $p = 0.018$) and total lung capacity (TLC) (79.5 ± 19.1 vs. 102.9 ± 14.8, $p = 0.029$) were significantly lower in the abnormal AAT group than in the normal AAT group. However, at 12 months, only TLC values (75.0 ± 12.2 vs. 104.9 ± 20.1, $p = 0.041$) showed a significant decrease in the abnormal AAT group.

For allele genotyping and respiratory function variables at 6 months, significant differences were found in the carbon monoxide transfer by a single breath (DLCO) ($p = 0.045$) and the distance covered in the 6MWT ($p = 0.040$). The analysis of multiple comparisons (Table S4 in the supplementary material) showed differences between the groups with the Pi*MM and Pi*MZ genotypes for TLC (mean difference 28.7 ± 10.6; $p = 0.023$), DLCO (mean difference 41.96 ± 15.5; $p = 0.024$) and the distance covered in the 6MWT (mean difference 210.2 ± 74.9 m, $p = 0.017$).

4. Discussion

Published reports issued in the weeks or months after discharge from hospital for COVID-19 pneumonia describe patients with varying degrees of persistent symptoms and radiological and functional abnormalities [22,23]. In the present study, risk factors associated with impaired lung function in patients who were hospitalised for COVID-19 were identified: a higher Charlson index, severe pneumonia with anemia, need for corticosteroid boluses of methylprednisolone, admission to the ICU. Our results are in line with

those published to date on risk factors associated with a worse clinical course, acute respiratory distress syndrome (ARDS) and death during hospitalization [24,25]. In this study, high levels of haemoglobin, lymphocytes, ferritin, albumin and troponin significantly reduced the risk of impaired lung function. The elevation of troponin and ferritin has previously been reported as a risk factor for poor clinical outcome, in contrast to our results. One explanation for their protective role may be the fact that these parameters increase early in cases with a poor clinical course and development of the inflammatory cascade thus potentially allowing early diagnosis and management.

We also found that close to one third of the patients (n = 49; 31.2%) continued to have impaired lung function at 12 months. The most affected functional parameters being FEV1 and DLCO. To date, there are studies that report on short- and medium-term functional outcomes [22,26]. However, studies evaluating one-year results have only recently become available. Wu et al. described persistent physiological (DLCO: 88% reduction of predicted) and radiographic (24% of patients) abnormalities in some patients 12 months after discharge for COVID-19 treatment [26]. Liu et al. reported physiological, laboratory, radiological or electrocardiogram abnormalities, with those related to renal, cardiovascular and liver function being particularly common, in patients who recovered from COVID-19 up to 12 months post-discharge [27]. Huang et al. reported 30% of patients with dyspnoea and 26% of patients with anxiety or depression at the 12-month visit among COVID-19 survivors [28]. In general for our study, all the functional variables improved from 6 to 12 months. However, only TLC and the distance covered in metres in the 6MWT reached statistical significance. Both variables showed a statistically significant decrease despite improvement in lung volumes. This may be due to persistent muscle weakness, which can cause dyspnoea on exertion and limit performance in this test.

With regard to AAT levels, two patients were in the abnormal range (both below normal), one with normal and one with impaired lung function. The mean level of AAT was similar in patients with normal and impaired lung function (124.4 ± 15.5 and 129.1 ± 18.2, respectively). However, there were 28 patients with normal levels of AAT and CRP >5 mg/L. It is worth noting that AAT is an acute-phase reactant and its plasma levels have been shown to increase 2–3 times in response to inflammatory or infectious stimuli, similar to CRP [20]. Complete genome sequencing was requested for these patients, but there were no pathogenic variants found.

The allelic variants of AAT in our patients were distributed in a similar way to the general population [29]; Pi*MM being predominant (131 patients: 83.4%) and deficient genotypes being far less common (Pi*MS 22 patients: 14.6%, Pi*MZ 2 patients: 1.3% and Pi*M/P Lowell 1 patient: 0.6%). Significant differences between the groups with normal and impaired lung function at 12 months were seen only in the normal genotype (Pi*MM). However, it should be noted that the two Pi*MZ cases and half of the Pi*MS cases were in the group of patients with impaired lung function. Moreover, the analysis of multiple comparisons revealed that there were statistically significant differences between PiMM and PiMZ genotypes in the patients with normal pulmonary function regarding DLCO and TM6M. In other study, the most common mild AATD genotypes were associated neither with increased SARS-CoV-2 infection rates nor with increased SARS-CoV-2 fatalities. The numbers of patients with severe AATD cases were too low to allow definitive conclusions [30].

With this study being single-centre and observational, there is a potential location bias. However, our study does have several strengths. It has a large sample size and included patients with moderate or severe COVID-19 with previous hospitalization who continue to have long-term functional sequelae. Many characteristics that influenced their recovery had not previously been reported.

In conclusion, our study found that close to one third of COVID-19 patients still showed impaired lung function 12 months after infection. The presence of a high Charlson index, severe pneumonia with anaemia, need for corticosteroid boluses, admission to the

ICU and low AAT levels or Pi*MZ deficiency allele variants predisposed patients to impaired lung function at 12 months after hospitalisation due to COVID-19.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/healthcare10122341/s1, Figure S1: Summary of the characteristics of the patients included in the study, Figure S2: Laboratory parameters of patients on admission, during hospitalization and at the 12-month follow-up, Figure S3: , Figure S4: Number and proportion of hospitalized patients with normal or impaired lung function at 12 months with plasma determination of alpha-1 antitrypsin (AAT), allelic genotyping (Pi*MM, Pi*MS, Pi*MZ and Pi*MP Lowell) and C-reactive protein (CRP) > 5 mg/dl.

Author Contributions: C.M.-G. designed and conducted the study with assistance from B.M.J.-R., J.G.-F. and E.M.T.-I.; B.M.J.-R. collected the demographic, clinical, psychological and pulmonary function test data in follow-up consultation supported by A.D.R.-O. and E.M.T.-I.; B.M.J.-R. captured and processed the HRCT images; J.G.-F. processed the laboratory indices; E.M.T.-I. and B.M.J.-R. analysed the data in SPSS; C.M.-G., B.M.J.-R., E.M.R.-U. and E.M.T.-I. wrote the manuscript; and all authors contributed to the discussion and interpretation of the results. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was carried out in accordance with the requirements stipulated in the Declaration of Helsinki (Tokyo revision, October 2004) and Spanish Organic Law 15/1999 on the Protection of Personal Data. The study was approved by the Hospital's Ethics Committee, assigning it the internal code 1017-N-20.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: The authors declare that they have no other competing interests.

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