UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE MEDICINA

TESIS DOCTORAL

Manifestaciones oftalmológicas asociadas a la enfermedad por Coronavirus (COVID-19)

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Noemi Güemes Villahoz

Directores

Carmen Dora Méndez Hernández
José María Martínez de la Casa
Julián García Feijoo

Madrid

UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE MEDICINA

TESIS DOCTORAL

MANIFESTACIONES OFTALMOLÓGICAS ASOCIADAS A LA ENFERMEDAD POR CORONAVIRUS (COVID-19)

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

Noemi Güemes Villahoz

DIRECTORES

Carmen Dora Méndez Hernández
José María Martínez de la Casa
Julián García Feijoo

UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE MEDICINA

PROGRAMA DE DOCTORADO EN CIENCIAS DE LA VISIÓN

TESIS DOCTORAL

MANIFESTACIONES OFTALMOLÓGICAS ASOCIADAS A LA ENFERMEDAD POR CORONAVIRUS (COVID-19)

Noemí Güemes Villahoz

DIRECTORES:
Carmen Dora Méndez Hernández
José María Martínez de la Casa
Julián García Feijoo

MADRID, 2022

AGRADECIMIENTOS

AGRADECIMIENTOS

La pandemia COVID-19 ha tenido y sigue teniendo un importante impacto a nivel sanitario, social y económico, siendo la investigación científica clave en el abordaje de un tema de enorme complejidad. Gran parte de este trabajo se ha desarrollado durante la crisis sanitaria, lo que ha obligado a trabajar en unas condiciones excepcionales y, esperemos irrepetibles.

Todavía recuerdo aquella llamada telefónica que hice a mi inseparable compañera, la Dra. Bárbara Burgos, donde un 17 de marzo de 2020, la proponía entrar en las habitaciones de los pacientes ingresados con COVID-19 del Hospital Clínico San Carlos, para obtener una muestra de lágrima y analizarla mediante PCR, con el objetivo de conocer la presencia del virus en el ojo y entender mejor su transmisión a través de la superficie ocular. Aunque pudiera parecer que en ese contexto "no tocaba arriesgarse", ambas pensamos que "sí tocaba" y, de verdad sentimos que teníamos un proyecto entre manos que nos motivaba y nos daba fuerzas en aquellos momentos tan difíciles para todos. A la Dra. Burgos, agradezco su apoyo incondicional en todos los proyectos de esta investigación. Su empuje y dedicación han sido fundamentales para el desarrollo de este trabajo. Eran tantos los interrogantes en aquel momento… que no dejaban de surgir más ideas y más proyectos, así que pronto se incorporó al equipo la Dra. Beatriz Vidal. Agradecer a ella también, toda su ayuda, todas esas tardes libres, fines de semana…. en medio de un estado de alarma, investigando y trabajando para llegar a resultados lo antes posible. Me llevo no sólo la experiencia que compartimos al trabajar en una situación tan excepcional, si no a unas buenas compañeras y amigas.

Sin duda alguna, hay una figura clave que ha intervenido en todos y cada uno de los proyectos COVID-19 que, desde marzo de 2020 hasta la fecha actual, el servicio de

oftalmología del Hospital Clínico San Carlos está llevando a cabo. Y esa figura es el jefe de servicio y codirector de esta Tesis Doctoral, Prof. Julián García Feijoo. Cuando el proyecto surgió en marzo de 2020, pensé que, si proponía algo así en ese momento tan crítico a un jefe de servicio que tenía otros 1000 asuntos con los que lidiar en esa fecha, no me sorprendería si me respondiera algo como… "no es el momento, más adelante". Pero lejos de responder eso, me apoyó, confió y creyó en la investigación. ¡Gracias de verdad! Gracias también a la Dra. Carmen Dora Méndez, quien siempre está ahí para todo con disposición y excepcional hacer, gracias por tu ánimo y empuje desde el inicio hasta el final de la Tesis. Gracias al Prof. Martínez de la Casa por su profesionalidad y sus magníficos consejos, por ser una inspiración personal y profesional. He sido muy afortunada en poder contar con los directores de esta Tesis Doctoral.

No puedo olvidar al resto de compañeros tanto residentes como especialistas del servicio de oftalmología del HCSC. Dr. Fernández Vigo, Dr. Donate, Dr. López Guajardo, Dra. Gómez de Liaño, Dr. Sáenz Francés, Dr. Diaz Valle…. Y todos aquellos que, aunque no nombre, no son por ello menos importantes. A todos y cada uno de ellos, GRACIAS.

Gracias a mis amigas de toda la vida, Cristina, Noelia, Pilar, María, Vanesa, Raquel, Bea… por escucharme, aguantarme, ¡por estar siempre! Soy una "suertuda".

Y dejo para el final lo más importante, mi familia. A mi marido, mi mejor amigo y mi mayor apoyo, Rodrigo, gracias por sacar la mejor versión de mí misma. A mis hijos, Clara, Alba y Martín, a quienes he robado los más valioso, tiempo con ellos. Espero inculcaros los valores que en su día a mí me inculcó mi padre, a quien hecho tanto de menos un día como hoy. A mi madre, Chus, que tira del carro de todos, sin preguntar y sin pedir nada a cambio. A mis hermanos, Alvar y Tania, pieza triangular de una familia unida. A mis abuelos, en especial a mi yaya. A todos mis tíos, tan presentes en mi vida.

En definitiva, gracias a mi grande y fantástica familia que me ha enseñado tantas cosas del trabajo, los valores de vida y la nobleza castellana. Gracias a los que están y a los que ya no están. GRACIAS PAPA, espero que si estás conduciendo tu moto entre las nubes me sonrías desde allí, porque en el medio del invierno he encontrado en ti, ¡un verano invencible!

LISTADO DE ABREVIATURAS

SARS-CoV-2	Coronavirus del Síndrome Respiratorio Agudo Severo-2
COVID-19	Enfermedad por Coronavirus-2019
ARN	Ácido Ribonucleico
OMS	Organización Mundial de la Salud
CDC	Centros de Control y Prevención de Enfermedades
ACE2	Enzima Convertidora de Angiotensina-2
TMPRSS-2	Proteasa Transmembrana de Serina-2
RT-PCR	Reacción en Cadena de la Polimerasa con transcripción inversa en tiempo real
Ct	Cycle threshold - Ciclo de umbral de positividad
OCT	Tomografía de coherencia óptica
OCTA	Angiografía por Tomografía de coherencia óptica
DD	Dímero D
CFNR	Capa de fibras nerviosas de la retina
CCG	Capa de células ganglionares
CPI	Capa plexiforme interna
DV	Densidad vascular
IF	Índice de flujo

ÍNDICE

INDICE

0 ORGANIZACIÓN GENERAL DE LA TESIS

La presente Tesis sigue la modalidad de Tesis en formato publicaciones, según artículo 10.3 del Real Decreto 99/2011, de 28 de enero (BOE 10/02/2011) que regula los estudios de doctorado en la Universidad Complutense de Madrid.

A continuación, se especifican los cinco artículos que constituyen el cuerpo de la tesis:

- **Güemes-Villahoz N,** Burgos-Blasco B, Arribi-Vilela A, Arriola-Villalobos P, Rico-Luna CM, Cuiña-Sardiña R, Delgado-Iribarren A, García-Feijoó J. Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19? J Med Virol. 2021 Jan;93(1):383-388. doi: 10.1002/jmv.26219.
- **Güemes-Villahoz N**, Burgos-Blasco B, García-Feijoó J, Sáenz-Francés F, Arriola-Villalobos P, Martinez-de-la-Casa JM, Benítez-Del-Castillo JM, Herrera de la Muela M. Conjunctivitis in COVID-19 patients: frequency and clinical presentation. Graefes Arch Clin Exp Ophthalmol. 2020 Nov;258(11):2501-2507. doi: 10.1007/s00417-020-04916-0.
- Burgos-Blasco B, **Güemes-Villahoz N**, Vidal-Villegas B, Martinez-de-la-Casa JM, Donate-Lopez J, Martín-Sánchez FJ, González-Armengol JJ, Porta-Etessam J, Martin JLR, Garcia-Feijoo J. Optic nerve and macular optical coherence tomography in recovered COVID-19 patients. Eur J Ophthalmol. 2021 Mar 15:11206721211001019. doi: 10.1177/11206721211001019.
- **Güemes-Villahoz N**, Burgos-Blasco B, Vidal-Villegas B, Donate-López J, de la Muela MH, López-Guajardo L, Martín-Sánchez FJ, García-Feijoó J. Reduced macular vessel density in COVID-19 patients with and without associated thrombotic events using optical coherence tomography angiography. Graefes

Arch Clin Exp Ophthalmol. 2021 Aug;259(8):2243-2249. doi: 10.1007/s00417-021-05186-0.

- **Güemes-Villahoz N**, Burgos-Blasco B, Vidal-Villegas B, Donate-López J, Martín-Sánchez FJ, Porta-Etessam J, López-Guajardo L, Martín JLR, González-Armengol JJ, García-Feijoó J. Reduced retinal vessel density in COVID-19 patients and elevated D-dimer levels during the acute phase of the infection. Med Clin (Barc). 2021 Jan 28:S0025-7753(21)00014-2. doi: 10.1016/j.medcli.2020.12.006.

Atendiendo a la regulación actual del Doctorado de la Universidad Complutense de Madrid, la presente Tesis se ha estructurado en los siguientes apartados: Introducción, Hipótesis y Objetivos, Compendio de los artículos publicados que constituyen el trabajo, Discusión, Conclusiones y Bibliografía. No contiene los apartados Material y Métodos y Resultados, que han sido sustituidos por los cinco artículos publicados, de acuerdo con la regulación actual de las Tesis en formato de publicaciones.

En los anexos administrativos de la Tesis se aportan varios documentos obligatorios para la presentación de la Tesis, como los informes de los directores y las autorizaciones de las editoriales, copyright para la inclusión de los artículos e imágenes.

1. RESUMEN

1 RESUMEN

Introducción: La infección por el coronavirus del síndrome respiratorio agudo severo 2 (SARS-CoV-2), responsable de la enfermedad por coronavirus 2019 (COVID-19), puede afectar a prácticamente todos los órganos, incluidos los ojos. Se ha detectado la presencia ARN de SARS-CoV-2 en lágrima y exudado conjuntival. También se han observado manifestaciones oculares tanto en el segmento anterior como en el segmento posterior, pudiendo aparecer tanto durante la fase aguda de la infección como tras la recuperación de la enfermedad. Además de estos cambios clínicos, se han observado cambios subclínicos en el grosor de las capas de la retina detectables mediante tomografía de coherencia óptica (OCT), y alteraciones en la circulación retiniana cuantificables mediante angiografía por OCT (OCTA).

Objetivos: El objetivo principal de esta Tesis se centra en el estudio de la afectación oftalmológica de la COVID-19. Para ello, se ha estudiado la presencia de ARN de SARS-CoV-2 en lágrima y exudado conjuntival, las características clínicas y la frecuencia de la conjuntivitis, y la afectación del segmento posterior mediante exploración del fondo de ojo, OCT y OCTA en pacientes COVID-19.

Métodos y Resultados: En la presente Tesis se presentan cinco estudios de investigación, todos ellos realizados en pacientes con infección confirmada por SARS-CoV-2. En el primer estudio, se ha investigado la presencia de ARN de SARS-CoV-2 en la superficie ocular mediante la realización de prueba RT-PCR de lágrima y exudado conjuntival en un grupo de pacientes COVID-19 con conjuntivitis y en un grupo COVID-19 sin conjuntivitis. Se detectó ARN de SARS-CoV-2 en dos pacientes (2/36). En cada uno de los grupos, se detectó el virus en un paciente respectivamente (1/18). Por tanto, se encontró la misma tasa de resultados positivos en el grupo con conjuntivitis (5.5%) que en el grupo sin conjuntivitis (5.5%).

En el segundo trabajo se incluyeron 301 sujetos ingresados por COVID-19. De ellos, 35 pacientes (11,6%) fueron diagnosticados de conjuntivitis aguda. No se encontró ninguna relación entre la gravedad de la COVID-19 y la presencia de conjuntivitis (p = 0,17). Sin embargo, la conjuntivitis fue más frecuente en los varones con COVID-19 moderado y en las mujeres con COVID-19 leve. La historia natural de la enfermedad parece ser de una conjuntivitis vírica, inespecífica y autolimitada que mejora sin espontáneamnete sin afectar a la visión, ni asociar complicaciones a corto plazo.

En el tercer trabajo se incluyeron 160 pacientes, 90 pacientes recuperados de COVID-19 y 70 controles históricos. Los pacientes con COVID-19 presentaron un aumento del grosor de la capa de fibras nerviosas retinianas (CFNR) peripapilar global (4,3; CI95% 0,8 a 7,7), nasal superior (6,9; CI95% 0,4 a 13,4) y nasal inferior (10,2; CI95% 2,4 a 18,1). A nivel macular, el grosor de la CFNR estaba disminuido en pacientes COVID-19 en volumen (-0,05; CI95% -0,08 a -0,02), cuadrante superior interno (-1,4; CI95% -2,5 a -0,4), nasal interno (-1,1; CI95% -1,8 a -0,3) y nasal externo (-4,7; CI95% -7,0 a -2,4); y el grosor de la capa de células ganglionares (CCG) estaba aumentado en volumen (0,04; IC95%: 0,01 a 0,07), superior externo (2,1; IC95%: 0,8 a 3,3), nasal externo (2,5; IC95%: 1,1 a 4). Además, los pacientes de COVID-19 con anosmia y ageusia presentaron un aumento del grosor de la CFNR peripapilar y de la CCG macular en comparación con los pacientes sin estos síntomas.

En otro trabajo, se realizó OCTA a 90 pacientes, 19 (20%) pacientes de COVID-19 que sufrieron eventos trombóticos (ET) asociados a la enfermedad, 47 (49,5%) pacientes de COVID-19 sin ET y 29 (30,5%) controles sanos. Los pacientes con COVID-19 presentaron una densidad vascular (DV) significativamente menor que los controles sanos: central (p = 0,003), anillo interno (p = 0,026), anillo externo (p = 0,001). La densidad de perfusión (DP) también estaba disminuida: anillo externo (p = 0,003), área

completa (p = 0,001). Sin embargo, no se encontraron diferencias en los parámetros de OCTA entre los pacientes de COVID-19 con y sin ET. Además, se correlacionó en otro trabajo los datos de OCTA con parámetros clínicos y laboratorio de pacientes COVID-19. Los pacientes con Dímero-D≥500ng/ml durante la infección por SARS-CoV-2 presentaron una disminución de la DV central (2,2; IC del 95%: 0,4-3,9) y DP (4,9; IC del 95%: 0,9-8,9) tras la fase aguda de la enfermedad. Estas diferencias no se documentaron en pacientes con LDH≥500 U/L, PCR≥10 mg/L e hipoxemia.

Discusión y Conclusiones: El SARS-CoV-2 está presente en la lágrima y exudado conjuntival, lo que pone de manifiesto el papel del ojo como posible vía de transmisión de la enfermedad. Sin embargo, la baja tasa de resultados positivos encontrados mediante RT-PCR de lágrima, sugiere que la realización de esta prueba tiene un limitado valor diagnóstico en la detección de la infección. La conjuntivitis es una manifestación ocular de la COVID-19 que puede aparecer en aproximadamente, 1 de cada 10 pacientes hospitalizados de COVID-19. Los pacientes recuperados de COVID-19 presentaron cambios en las capas de la retina detectables mediante OCT, además de una DV y DP disminuida comparado con controles sanos. Estos hallazgos subrayan el papel de la OCT y OCTA como posibles biomarcadores no invasivos de la disfunción inflamatoria y vascular relacionada con la infección por el SARS-CoV-2.

ABSTRACT

Introduction: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), responsible for coronavirus disease 2019 (COVID-19), may affect virtually all organs, including the eyes. SARS-CoV-2 RNA has been detected in tears and conjunctival swab. Ocular manifestations have also been observed in both the anterior and posterior segments and can appear both during the acute phase of infection and after recovery from the disease. In addition to these clinical changes, subclinical changes in the thickness of retinal layers detected by optical coherence tomography (OCT), and disturbances in retinal circulation measurable by OCT angiography (OCTA) have been observed.

Objectives: The main objective of this Thesis focuses on the study of the ophthalmologic involvement of COVID-19. For this purpose, the presence of SARS-CoV-2 RNA in tear and conjunctival exudate, clinical features and frequency of conjunctivitis, and posterior segment involvement by fundus examination, OCT and OCTA in COVID-19 patients have been studied.

Methods and Results: In the present Thesis, five research studies are presented, all of them performed in patients with confirmed SARS-CoV-2 infection. In the first study, the presence of SARS-CoV-2 RNA on the ocular surface was investigated by performing RT-PCR testing of tear and conjunctival exudate in a group of COVID-19 patients with conjunctivitis and in a COVID-19 group without conjunctivitis. SARS-CoV-2 RNA was detected in two patients (2/36). In each of the groups, virus was detected in one patient respectively (1/18). Therefore, the same rate of positive results was found in the group with conjunctivitis (5.5%) as in the group without conjunctivitis (5.5%).

The second study included 301 subjects admitted for COVID-19. Of these, 35 patients (11.6%) were diagnosed with acute conjunctivitis. No relationship was found between

the severity of COVID-19 and the presence of conjunctivitis (p = 0.17). However, conjunctivitis was more frequent in males with moderate COVID-19 and in females with mild COVID-19. The natural history of the disease appears to be a viral, nonspecific, self-limited conjunctivitis that improves rapidly without treatment, affecting neither vision nor short-term complications.

The third study included 160 patients, 90 patients recovered from COVID-19 and 70 historical controls. Patients with COVID-19 had increased global RNFL thickness peripapillary (4.3; CI95% 0.8 to 7.7), superior nasal (6.9; CI95% 0.4 to 13.4) and inferior nasal (10.2; CI95% 2.4 to 18.1). Macular RNFL thickness was decreased in COVID-19 patients in volume (-0.05; CI95% -0.08 to -0.02), upper inner quadrant (-1.4; CI95% -2.5 to -0.4), inner nasal (-1.1; CI95% -1.8 to -0.3), and outer nasal (-4.7; CI95% -7.0 to -2.4); and CCG thickness was increased in volume (0.04; CI95%: 0.01 to 0.07), external superior (2.1; CI95%: 0.8 to 3.3), external nasal (2.5; CI95%: 1.1 to 4). Furthermore, COVID-19 patients with anosmia and ageusia had increased peripapillary RNFL thickness and macular GCC compared to patients without these symptoms.

In another paper, OCTA was performed on 90 patients, 19 (20%) COVID-19 patients who experienced disease-associated thrombotic events (TE), 47 (49.5%) COVID-19 patients without TE and 29 (30.5%) healthy controls. COVID-19 patients had significantly lower vessel density (VD) than healthy controls: central (p = 0.003), inner ring (p = 0.026), outer ring (p = 0.001). Perfusion density (PD) was also decreased: outer ring (p = 0.003), whole area (p = 0.001). However, no differences in OCTA parameters were found between COVID-19 patients with and without TE. In addition, OCTA data were correlated in another paper with clinical and laboratory parameters of COVID-19 patients. Patients with D-Dimer≥500ng/ml during SARS-CoV-2 infection had decreased central DV (2.2; 95% CI 0.4-3.9) and PD (4.9; 95% CI 0.9-8.9) after the acute phase of

the disease. These differences were not documented in patients with LDH≥500 U/L, CRP≥10 mg/L and hypoxemia.

Discussion and Conclusions: SARS-CoV-2 is present in tears and conjunctival secretions, highlighting the role of the eye as a possible route of spread of the infection. However, the low rate of positive results found by tear RT-PCR suggests that this test has limited diagnostic value in detecting infection. Conjunctivitis is an ocular manifestation of COVID-19 that may occur in approximately 1 in 10 hospitalized COVID-19 patients. Recovered COVID-19 patients had changes in retinal layers thickness detectable by OCT, in addition to decreased DV and PD compared to healthy controls. These findings emphasize the potential role of OCT and OCTA as possible noninvasive biomarkers of inflammatory and vascular dysfunction related to SARS-CoV-2 infection.

3. INTRODUCCIÓN

2 INTRODUCCIÓN

El 31 de diciembre de 2019, la Comisión Municipal de Salud y Sanidad de la ciudad de Wuhan (provincia de Hubei, China) notificó a la Organización Mundial de la Salud (OMS) la existencia de un brote de neumonía de etiología no filiada en la ciudad de Wuhan. El agente responsable de este brote fue identificado y publicado por las autoridades chinas el día 7 de enero de 2020, encontrando como nexo común entre los casos afectados la exposición a un mercado mayorista de marisco y animales vivos en la citada ciudad de Wuhan. Este reporte notificaba el hallazgo de un nuevo virus de la familia Coronaviridae, al que inicialmente se le denominó 2019-nCoV y, posteriormente, fue rebautizado por el Comité Internacional de Taxonomía de Virus (ICTV) con el nombre coronavirus del síndrome respiratorio agudo severo 2 (SARS-CoV-2).[1] El 11 de febrero de 2020, la OMS denominó oficialmente a esta enfermedad como Enfermedad por Coronavirus-2019 (COVID-19),[2] y, posteriormente, el día 11 de marzo de 2020, este mismo organismo declaró el status de pandemia mundial.[3]

La infección por SARS-CoV-2, en el momento de la redacción de este documento, ha sido responsable de más 476 millones de casos confirmados de COVID-19, incluyendo más de 6 millones de víctimas mortales a nivel mundial,[4] además de tener un extraordinario impacto socioeconómico en todo el mundo. Concretamente, en España se han reportado hasta la fecha más de 11 millones de casos confirmados de COVID-19 y 102.119 muertes.[4]

A pesar de que la COVID-19 afecta principalmente al sistema respiratorio, cada vez hay más evidencia de que puede afectar a prácticamente todos los órganos, incluidos los ojos.[5–7] Esta Tesis en formato publicaciones ha estudiado la presencia de ARN de SARS-CoV-2 en lágrima y exudado conjuntival, las características clínicas y la frecuencia de la conjuntivitis como principal manifestación oftalmológica de la COVID-19, así como la

afectación del segmento posterior mediante exploración del fondo de ojo, tomografía de coherencia óptica (OCT) y angiografía por tomografía de coherencia óptica (OCTA).

2.1 Características del SARS-CoV-2

El virus SARS-CoV-2 pertenece a la subfamilia Coronaviridae (orden Nidovirales) que a su vez contiene cuatro géneros: Alphacoronavirus, Betacoronavirus, Gammacoronavirus y Deltacoronavirus.[8] El SARS-CoV-2 es un Betacoronavirus, al igual que sus predecesores, el coronavirus del síndrome respiratorio agudo (SARS-CoV) y el coronavirus del síndrome respiratorio de Oriente Medio (MERS-CoV). Por su parte, el SARS-CoV fue responsable del brote epidémico identificado en China en el año 2002,[9,10] y el MERS-CoV, identificado en el año 2012, causó un brote que afectó mayoritariamente a Arabia Saudí.[11]

El SARS-CoV-2 es un virus ARN monocatenario de sentido positivo (conocido por sus siglas en inglés como +ssRNA, single-stranded positive-sense RNA). Estructuralmente los coronavirus son virus esféricos que constan de 4 proteínas estructurales: la proteína S (spike protein), la proteína E (envelope), la proteína M (membrane) y la proteína N (nucleocapsid), y de dieciséis proteínas no estructurales (nsp1-16). Figura 1. La proteína S otorga al virus su característica apariencia de corona y, además, contiene el dominio de unión al receptor celular, siendo por tanto la proteína determinante del tropismo del virus. El tamaño del genoma del SARS-CoV-2 es de aproximadamente 29,9 kb, y fue secuenciado por Lu y colaboradores en Enero de 2020,[12] quienes encontraron que estaba estrechamente relacionado (con un 88% de identidad) con dos coronavirus derivados de murciélagos similares al SARS, el bat-SL-CoVZC45 y el bat-SL-CoVZXC21, recogidos en 2018 en Zhoushan, China. Además, este mismo estudio encontró que su homología de secuencia era menor con el virus SARS-CoV (79%) y el virus MERS-CoV (50%). Sin

embargo, el análisis filogenético reveló que el dominio de unión al receptor del SARS-CoV-2 era similar al SARS-CoV. De modo que ambos utilizan la enzima convertidora de angiotensina 2 (ECA-2) como principal receptor celular.

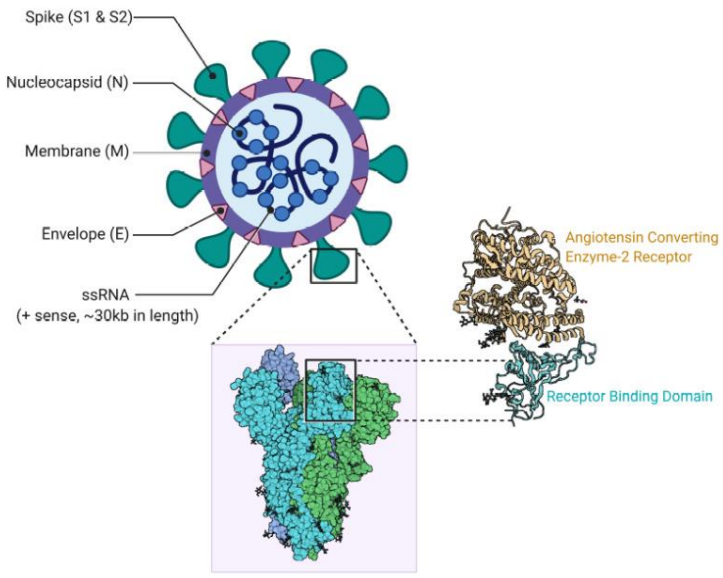

Figura 1: Estructura del SARS-CoV-2. De: Cascella M et al. Features, Evaluation, and Treatment of Coronavirus (COVID-19). StatPearls Publishing.

2.2 Fuente de infección: origen del SARS-CoV-2

El origen del SARS-CoV-2 continúa siendo una incógnita en muchos aspectos. A pesar de que la teoría de un probable origen zoonótico a través de un huésped intermedio parece ser la más aceptada, la fuente concreta de la infección no se conoce con exactitud por el momento. Con el fin de conocer el origen y transmisión del SARS-CoV-2, la OMS envió un grupo de expertos internacionales y chinos (17 científicos internacionales y 17 científicos chinos) a la ciudad de Wuhan entre el 14 de enero y el 10 de febrero de 2021. El equipo de la misión examinó cuatro posibles escenarios y emitió el siguiente informe acerca de la probabilidad de cada uno de ellos[13]:

1. La propagación zoonótica directa se considera una vía de posible a probable.

2. La introducción a través de un huésped intermedio se considera una vía de probable a muy probable.
3. La introducción a través de productos de la cadena de frío/alimentos se considera una vía posible.
4. La introducción a través de un incidente de laboratorio se considera una vía extremadamente improbable.

Según este informe, la vía más probable sería la introducción a través de un anfitrión intermedio. Esta hipótesis apoya que el SARS-CoV-2 se transmitiría desde un reservorio animal a un anfitrión intermedio animal, y de éste se transmitiría al ser humano. El análisis del genoma sugiere que los murciélagos pueden ser la fuente del SARS-CoV-2, y que el pangolín podría ser uno de los hospedadores intermedios. Sin embargo, la distancia genética tanto del murciélago como del pangolín, así como el hecho de que ambos animales entran en contacto con los seres humanos con poca frecuencia, sugiere que otros anfitriones intermedios han intervenido en la cadena de transmisión, aunque por el momento se desconoce cuáles.

Otra vía de transmisión que ha generado gran controversia ha sido la introducción a través de un accidente de laboratorio. Según esta teoría, el SARS-CoV-2 se introduciría a través de un incidente de laboratorio, como un escape de material infectivo al exterior o una infección accidental de algún trabajador. No obstante, esta hipótesis parece ser la menos probable según el informe de la OMS.[13]

2.3 Mecanismos de transmisión de la infección humano-humano

2.3.1 Transmisión por secreciones respiratorias

La principal vía de transmisión del SARS-CoV-2 es la inhalación de las gotas y aerosoles respiratorios emitidos por un enfermo o el contacto directo de estas secreciones con las

mucosas de las vías respiratorias y la conjuntiva del susceptible. El contacto indirecto a través de las manos u objetos contaminados es otra posible vía.[14] En cuanto a la transmisión de la infección por SARS-CoV-2 mediante aerosoles, esta vía continua siendo objeto de controversia entre distintos autores.[15,16] Un aerosol es un conjunto de partículas sólidas o líquidas suspendidas en un gas, como el aire.[17] La evidencia actual clasifica las partículas respiratorias según su tamaño en gotas y en aerosoles. El tamaño de corte comúnmente aceptado entre las gotas y los aerosoles es de 5 μm, aunque varía considerablemente entre los estudios.[18,19] La OMS y los Centros de Control y Prevención de Enfermedades (CDC) establecieron que, las partículas de más de 5 μm se comportan como gotas, y las de menos de 5 μm como aerosoles, en su guía de prevención de la transmisión de agentes infecciosos en el ámbito sanitario,[20] aunque muchos autores consideran que estas guías deben ser revisadas.[15,19,21] Las personas liberan fluidos respiratorios durante la exhalación (al respirar en silencio, hablar, cantar, hacer ejercicio, toser o estornudar) en forma de gotas de distintos tamaños. El comportamiento aerodinámico de estas partículas puede ser diferente dependiendo del tamaño. No obstante, la capacidad de una partícula para permanecer en suspensión depende de muchos otros factores además del tamaño, como la velocidad con la que son expulsadas, las características del flujo de aire circundante (velocidad, turbulencia, dirección, temperatura y humedad relativa).[22] De ahí que sea difícil especificar un punto de corte a partir del cual una partícula queda suspendida en el aire.[21] Por este motivo, a efectos de describir la transmisión, el umbral de tamaño más aceptado para distinguir las gotitas de los aerosoles, en cuanto a su comportamiento físico y la vía de exposición, es de 100 μm.[22] Así, las gotas con un tamaño mayor de 100 micras tienen un comportamiento balístico. Estas partículas caen al suelo en cuestión de segundos o minutos y recorren una distancia máxima de aproximadamente dos metros. Por otro lado, las emisiones

respiratorias de tamaño inferior a 100 micras pueden permanecer suspendidas en el aire durante más tiempo (minutos u horas) y viajar una distancia superior a dos metros del emisor, pudiendo ser inhaladas por otra persona, incluso en ausencia de un emisor si aún persisten suspendidas en el aire. Hay varios ejemplos bien documentados en los que el SARS-CoV-2 parece haber sido transmitido mediante aerosoles.[23–25] Entre ellos, destacan un incidente de contagio descrito en un restaurante en China y otro durante un ensayo de coro en Washington DC,[24,25] donde se demostró que las personas sentadas a una distancia de más de 2 metros de la persona infectada fueron contagiadas con la enfermedad. Según los criterios establecidos por Jones y Brosseau en 2015, la transmisión por aerosol es plausible cuando (1) los aerosoles que contienen virus son generados por o una persona infectada, (2) el virus permanece viable en los aerosoles durante algún periodo de tiempo, y (3) los tejidos diana donde el virus inicia la infección son accesibles por el aerosol con suficiente carga viral. Basándose en la evidencia disponible hasta el momento en estudios empíricos y de laboratorio, la infección por SARS-CoV-2 parece cumplir estos criterios.[23–28] Adicionalmente, la OMS y los CDC han reconocido en informes recientes esta vía como una posible vía de transmisión en determinadas circunstancias.[14,29]

2.3.2 Transmisión por superficies contaminadas (fómites)

El riesgo de transmisión de la infección por fómites depende de múltiples factores, entre los que destacan la deposición de las partículas de virus expulsadas en las superficies que se ve afectada por el flujo de aire y la ventilación, la interacción con los factores ambientales como el calor y la evaporación, la carga viral, y el tiempo que transcurre entre el momento en que una superficie se contamina y el momento en que una persona toca la superficie.[30] Se han notificado algunos casos de COVID-19 atribuidos potencialmente a la transmisión por fómites.[31,32] Sin embargo, este tipo de transmisión

por fómites es difícil de probar con certeza, en parte porque no se puede descartar la transmisión respiratoria de personas asintomáticas. Por otro lado, a pesar de que se haya detectado la presencia del SARS-CoV-2 en diferentes superficies, se pone en duda la viabilidad y duración del virus en la superficie en condiciones reales.[33,34] Debido a los múltiples factores que entran en juego en esta ruta de transmisión, se considera que el riesgo relativo de transmisión por fómites del SARS-CoV-2 es bajo en comparación con otras vías como la transmisión por gotas o aire y el contacto directo.

2.3.3 Transmisión vertical

La trasmisión vertical a través de la placenta también se ha documentado,[35] aunque se considera que la transmisión de la infección madre-hijo se produce fundamentalmente tras el nacimiento del bebé por el contacto con las secreciones respiratorias de la madre.[36] Respecto a la lactancia materna, se ha detectado RNA de SARS-CoV-2 en muestras de leche materna de algunas pacientes con COVID-19.[37] Sin embargo, según una revisión sistemática reciente, no hay pruebas de la transmisión del SARS-CoV-2 a través de la leche materna[38]

2.3.4 Transmisión fecal-oral

En cuanto a la trasmisión fecal-oral, se ha detectado la presencia de SARS-CoV-2 en heces,[39] aunque todavía no se ha establecido la ruta exacta de transmisión fecal-oral. Se ha publicado una posible transmisión fecal-aerosol como origen de un brote comunitario de COVID-19 en un rascacielos en Guangzhou, China, donde tres familias que vivían en pisos alineados verticalmente y conectados por tubos de desagüe de los baños principales, fueron infectadas, sin encontrar otra vía de contacto alternativa que pudiera explicar este

brote.[40] A pesar de ello, no existe suficiente evidencia hasta la fecha para demostrar esta vía.

2.3.5 Superficie ocular y fluidos oculares como potencial vía de transmisión

Se ha detectado la presencia ARN de SARS-CoV-2 en lágrima y exudado conjuntival,[41,42] hecho que pone de manifiesto el papel de la superficie ocular como posible vía de transmisión de la infección. Uno de los primeros casos reportados a este respecto fue el de un médico que trabajaba en Wuhan.[43] Este médico atendió a pacientes con COVID-19 con mascarilla N95, pero sin protección ocular. A pesar de llevar mascarilla, contrajo la infección y presentó una marcada hiperemia conjuntival asociada, lo que planteó que la exposición al SARS-CoV-2 sin protección ocular adecuada podría ser una potencial vía de transmisión de la enfermedad.[43] Un estudio multicéntrico evaluó los factores de riesgo para la transmisión del SARS-CoV-2 de pacientes que requerían intubación a pesar del cumplimiento de los protocolos de control de infecciones.[44] Este trabajo encontró que el contacto ocular sin protección con secreciones de pacientes infectados era la variable más predictora para la transmisión de la infección en los trabajadores sanitarios. Esta observación puso de relieve la importancia de utilizar gafas de protección como parte integrante del equipo de protección individual.

El tropismo viral hacia el tejido ocular puede explicarse en parte por la presencia de receptores para el SARS-CoV-2 a nivel de la superficie ocular. El principal receptor de entrada celular del virus es la enzima convertidora de angiotensina-2 (ACE2).[45] La proteasa transmembrana de serina 2 (TMPRSS 2) participa en la escisión de la proteína S del SARS CoV-2, lo que favorece la entrada del virus a la célula huésped. [46] Estos receptores han sido identificados en una amplia gama de tejidos humanos, incluyendo la superficie ocular (córnea y conjuntiva)[47,48] y retina y coroides[49]. Por otro lado, el CD147,

también conocido como Basigin o inductor de metaloproteínas de la matriz extracelular, es una glicoproteína transmembrana que está asociada a la infección viral. Estudios recientes han encontrado una posible interacción entre el receptor de la célula huésped CD147 y la proteína S del SARS-CoV-2.[50] Mediante análisis inmunohistoquímico se ha detectado la presencia de CD147 en la córnea, la conjuntiva, la retina y el epitelio pigmentario de la retina.[51] Este mismo análisis encontró que la proteína CD147 también existe en forma soluble en la lágrima, el humor acuoso y el vítreo. Estos resultados proporcionan una base molecular para la propagación del SARS-CoV-2 a través de la superficie ocular.[52]

2.3.6 Otras posibles vías de transmisión

En cuanto a la transmisión sexual de la infección, a pesar de que se ha detectado el virus en el semen de algunos pacientes en la fase aguda o de convalecencia de la COVID-19,[53] actualmente no hay pruebas de que el virus se transmita a través del semen o de los fluidos vaginales, y por tanto, la mayoría de expertos coinciden en que esta vía es poco probable.[54] La presencia del SARS-CoV-2 en otros fluidos corporales, como la sangre y hemoderivados,[55,56] también generó controversia acerca de la posible transmisión sanguínea de la infección. Sin embargo, no hay ningún caso de transmisión sanguínea de la COVID-19 documentado hasta la fecha.

2.4 DINÁMICA DE LA TRANSMISIÓN DEL SARS-COV-2

2.4.1 Periodo de incubación y periodo infectivo

El período de incubación se define como el tiempo que transcurre entre la exposición inicial al virus y la aparición de los síntomas de la enfermedad. Este parámetro tiene un papel relevante en las medidas de control y prevención de enfermedades infecciosas,

incluyendo el cálculo del tiempo de cuarentena adecuado. Según un metaanálisis reciente, el periodo de incubación mediano de COVID-19 es de 5,1 días (IC 95% 4,5 a 5,8). y el 97,5% de los que desarrollan síntomas lo harán en los 11,5 días (IC, 8,2 a 15,6 días) siguientes a la infección.[57] Otro parámetro importante en el control de la infección es el intervalo serial, el cual se define como el intervalo de tiempo entre la aparición de los síntomas en el caso primario y el secundario. El intervalo serial medio de COVID-19 en numerosas observaciones epidemiológicas resultó menor que el periodo de incubación, lo que sugiere que una proporción sustancial de transmisión se produce en la fase presintomática.[58] El periodo infectivo se define como el periodo durante el cual un sujeto puede contagiar la infección. Puesto que el cultivo celular del SARS-CoV-2 para la detección de virus viable en muestras clínicas tiene una sensibilidad relativamente baja, la técnica molecular de la reacción en cadena de la polimerasa con transcripción inversa en tiempo real (RT-PCR) es habitualmente utilizada para el cálculo de este periodo.[59] Sin embargo, la RT-PCR permite detectar la presencia de ARN viral, sin que ello implique la detección de virus con capacidad de replicación. De hecho, se ha encontrado un número no despreciable de sujetos que presentan pruebas RT-PCR persistentemente o intermitentemente positivas durante semanas, sin que esto implique que estos individuos sean una fuente significativa de transmisión del SARS-CoV-2.[60] No obstante, la cantidad de ARN viral detectado mediante RT-PCR o carga viral ha mostrado tener una relativa correlación con la positividad de los cultivos virales.[61] De acuerdo con la evidencia existente hasta la fecha, la transmisión de la infección por SARS-CoV-2 se produce fundamentalmente desde los 2-3 días previos al inicio de los síntomas hasta 7-8 días después. En los casos más graves de COVID-19 parece que esta transmisión es más intensa y duradera.[62]

2.4.2 Número básico y efectivo de reproducción

El número básico de reproducción (R0) es un término epidemiológico que describe el número esperado de infecciones secundarias generadas por un caso. Al principio de la pandemia, las instituciones de salud pública y el público en general hacían referencia al R0 con frecuencia. Sin embargo, esta métrica a menudo se utiliza o se interpreta de forma incorrecta.[63] Para evaluar la dinámica a tiempo real de la transmisión de enfermedades infecciosas se suele utilizar el número reproductivo efectivo (Re). Esta métrica se emplea para evaluar la eficacia de las medidas preventivas de salud pública tomadas para una evitar la transmisión de una enfermedad y en su cálculo intervienen el número de casos, el intervalo serial y el momento de inicio de los síntomas.

2.5 DIAGNÓSTICO DE LA INFECCIÓN POR SARS-CoV-2

El diagnóstico de la infección juega un papel clave en el control de la propagación de la enfermedad. En la actualidad, existen diferentes pruebas para el diagnóstico de la infección por SARS-CoV-2, que incluyen: detección de RNA mediante RT-PCR o qRT-PCR (si se cuantifica en tiempo real), detección antígenos virales (Ag), detección de anticuerpos totales (Ac), detección anticuerpos IgM/IgA o IgG. Las pruebas de detección de Anticuerpos (Ac) rápidas (inmunocromatografía) o de alto rendimiento (ELISA) son pruebas indirectas, en las que se detectan Ac producidos en respuesta a la exposición al virus. Estas técnicas permiten la detección de Ac totales o Ac específicos (IgM, IgA o IgG) y, por lo tanto, no se emplean en el diagnóstico de la infección activa.

2.5.1 Pruebas diagnósticas de infección activa

Las pruebas diagnósticas de infección activa (PDIA) habitualmente empleadas son los Test rápidos de Antígeno (Test Ag) y la RT-PCR. La RT-PCR es una técnica molecular

de detección de material genómico por amplificación de ácidos nucleicos, y que, en la actualidad, se considera la técnica de referencia para el diagnóstico del SARS-CoV-2.[64] Esta prueba se puede llevar a cabo en diferentes tipos de muestras. No obstante, las muestras que por el momento ofrecen mayor rentabilidad y son recomendadas por los CDC son las nasofaríngeas, seguidas por las orofaríngeas.[65] Los genes diana habitualmente empleados con esta técnica son el gen E de la envoltura, el gen RpRd y el gen N de la nucleocápside.

La positividad para un único gen del SARS-CoV-2 se considera suficiente para confirmar el diagnóstico en lugares con circulación viral comunitaria. El momento más rentable para la obtención de la muestra ha sido extensamente estudiado.[64,66–68] La mayoría de los autores coinciden en que el periodo de máxima sensibilidad de la PCR para la detección del ARN del SARSCoV-2 es los primeros 7 días desde el inicio de los síntomas.[67,68] En el caso de asintomáticos y contactos estrechos, se recomienda realizar el test entre los días 5-7 días postexposición.[64,66] La sensibilidad de esta prueba varía, entre otros, en función del momento de obtención de la muestra, Así, una revisión sistemática reciente encontró que el porcentaje más alto de detección del virus de una muestra nasofaríngea se produjo entre los días 0 y 4 después de la aparición de los síntomas, con un 89% de sensibilidad (intervalo de confianza [IC] del 95%: 83 a 93), y que ésta descendió al 54% (IC del 95%: 47 a 61) después de 10 a 14 días.[68] En cuanto a su especificidad, es cercana al 100%. La cuantificación de la carga viral se estima mediante el ciclo de umbral de positividad – Cycle threshold (Ct). Este dato refleja el número de ciclos en una RT-PCR que se necesita para amplificar el ARN para detectarlo. De ahí que, el valor del Ct sea inversamente proporcional a la carga viral, es decir, un Ct bajo refleja una alta carga viral. No obstante, es importante recalcar que no es posible traducir directamente un valor Ct en grado o duración de la contagiosidad, ya que su valor puede variar en función del test

y el tipo y calidad de la muestra empleada entre otros factores.[69,70] A pesar de ello, se ha publicado que un valor umbral de Ct > 30-35 podría corresponder a un virus potencialmente no infectivo, siempre que se correlacione con la clínica, la gravedad y el estado de inmunodepresión del paciente.[70]

Los test de Ag se basan en la detección directa de las proteínas virales del SARS-CoV-2 (antígenos) en hisopos nasofaríngeos y otras secreciones respiratorias. Estas pruebas son también conocidas como pruebas rápidas de Ag, y ofrecen un diagnóstico menos costoso y más rápido que la PCR.[71] Este método es especialmente útil en cribados masivos, en la detección primaria de casos en individuos sintomáticos sospechosos de estar infectados, en individuos asintomáticos con alto riesgo de contraer la infección y en el rastreo de contactos entre otros. Su sensibilidad es variable[72–74]. Un meta-análisis reciente que incluyó 14 estudios con un total de 8624 participantes encontró una sensibilidad del 79% y una especificidad del 100%.[72] El análisis de subgrupos de los estudios que informaron de la recogida de muestras en los 7 días siguientes al inicio de los síntomas mostró una sensibilidad superior (95%) y una especificidad del 100%. En general, se considera que las pruebas de antígenos tienen una sensibilidad moderada y una especificidad alta para la detección del SARS-CoV-2, y que esta sensibilidad aumenta cuando la prueba se realiza en los 5-7 días siguientes al comienzo de los síntomas. En pacientes asintomáticos hay escasa evidencia, se ha reportado una sensibilidad del 44% en asintomáticos.[75] Los falsos negativos son por tanto uno de los principales inconvenientes de esta prueba diagnóstica, por lo que un resultado negativo no descarta la infección, y es recomendable realizar una prueba PCR en caso de alta sospecha diagnóstica.[76]

2.5.2 Detección del SARS-CoV-2 en lágrima y exudado conjuntival

El ARN del SARS-CoV-2 se ha detectado en fluidos oculares de pacientes con COVID-19 tanto con, como sin conjuntivitis.[77–79] Sin embargo, la recogida de lágrimas y secreciones oculares para la detección del SARS-CoV-2 parece tener un valor diagnóstico limitado. Un estudio reciente evaluó las lágrimas y las muestras conjuntivales de 30 pacientes con neumonía por COVID-19. De ellos, el único paciente que presentó un resultado positivo en la RT-PCR de exudado conjuntival, fue un paciente que manifestó conjuntivitis como afectación ocular asociada a la enfermedad.[77] A la luz de estos resultados, se sugirió inicialmente que el valor diagnóstico de la prueba podría ser mayor en los pacientes con conjuntivitis que en aquellos que no manifiestan signos ni síntomas de conjuntivitis asociada a la infección por SARS-CoV-2. Sin embargo, un estudio posterior, que incluyó a 121 pacientes afectados por la enfermedad, encontró resultados positivos en la RT-PCR de exudado conjuntival de un paciente con conjuntivitis y dos pacientes sin conjuntivitis. Por tanto, la proporción encontrada de resultados positivos para la detección del SARS-CoV-2 en la muestra de exudado conjuntiva fue del 2,5% (3/121).[79] El empleo de muestras de secreciones oculares para la detección de ARN de SARS-CoV-2, parece tener un valor diagnóstico limitado debido a la baja tasa de resultados positivos encontrados. En base a estos resultados, nuestro grupo de trabajo realizó un estudio que incluyó a pacientes ingresados con COVID-19 en el Hospital Clínico San Carlos de Madrid en abril de 2020. En este trabajo, que forma parte del cuerpo de esta tesis,[80] se estudió la presencia de ARN de SARS-CoV-2 en fluidos oculares en pacientes con y sin conjuntivitis asociada a la COVID-19, con el objetivo de evaluar el valor diagnóstico de la realización de PCR de lágrima y exudado conjuntival en ambos grupo de pacientes.

3 MANIFESTACIONES CLÍNICAS DE LA COVID-19

Las manifestaciones clínicas más frecuentes de la COVID-19 son de tipo respiratorio. No obstante, se ha reportado la afectación de prácticamente todos los órganos asociada a la infección.[6,81–83] El registro de la Sociedad Española de Medicina Interna (SEMI) ha publicado las características clínicas de 15.111 pacientes con COVID-19 atendidos en 150 hospitales en España.[83] Los síntomas más frecuentes fueron fiebre (84,2%), tos (73,5%), disnea (57,6%) y astenia (43,6%). Otros síntomas menos frecuentes fueron diarrea (23.7%), anorexia (19.6%) y anosmia (7.1%). Estos resultados coinciden con otros estudios similares publicados hasta la fecha.[6,84,85]

En cuanto a la prevalencia de pacientes asintomáticos con infección por SARS-CoV-2, desde el origen de la pandemia se han reportado cifras variables.[86–90] No obstante, la mayoría de los estudios recientes han encontrado una prevalencia entre el 40.5% y el 48% de pacientes asintomáticos con la infección SARS-CoV-2.

A continuación, se describen las manifestaciones clínicas de la COVID-19 agrupadas por órganos y sistemas:

3.1 Manifestaciones Pulmonares

La afectación pulmonar es sin duda la más frecuentemente encontrada en pacientes con COVID-19. Síntomas como tos, expectoración, disnea e insuficiencia respiratoria son habituales en pacientes atendidos con COVID-19. Las opacidades en vidrio esmerilado y la neumonía bilateral en la tomografía axial computarizada (TAC) de tórax son los hallazgos más relevantes en pacientes ingresados con la enfermedad.[82]

3.2 Manifestaciones Gastrointestinales

Las manifestaciones gastrointestinales asociadas a la infección se han publicado en un porcentaje variable de individuos con COVID-19 (11.4-61.1%), con una aparición y gravedad variables.[91,92] La mayoría de los síntomas gastrointestinales asociados a la COVID-19 son leves y autolimitados. Estos incluyen anorexia, diarrea, náuseas, vómitos y dolor o malestar abdominal. También se ha reportado patología gastrointestinal más grave en una minoría de pacientes, como pancreatitis aguda, apendicitis aguda, obstrucción intestinal, isquemia intestinal, hemoperitoneo o síndrome compartimental abdominal.[91]

3.3 Manifestaciones Hematológicas

Es frecuente que los pacientes atendidos con COVID-19 presenten parámetros de laboratorio alterados. En el registro español de la SEMI, los parámetros de laboratorio más frecuentemente afectados fueron los niveles elevados de ferritina (73,5%), lactato deshidrogenasa (73,9%) y dímero D (63,8%), así como la linfopenia (52,8%).[83] Además, algunos de estos parámetros son considerados biomarcadores pronósticos asociados con COVID-19 severo, como la leucocitosis, la neutrofilia, la elevación de la proporción entre neutrófilos y linfocitos, la trombocitopenia, el tiempo de tromboplastina parcial activada (APTT), el dímero D, los niveles de lactato deshidrogenasa (LDH), ferritina sérica y proteína C reactiva, entre otros.[93,94] En lo referente a las complicaciones tromboembólicas, se han descrito casos de infarto cerebral, isquemia cardiaca, muerte súbita, embolismos y trombosis venosa profunda. También se ha observado una mayor incidencia de sangrados a múltiples niveles.[94]

3.4 Manifestaciones Neurológicas

La afectación neurológica asociada al COVID-19 se ha descrito tanto a nivel del sistema nervioso central como del sistema nervioso periférico. El dolor de cabeza es uno de los síntomas más frecuentemente observados dentro de la afectación neurológica central.[95] Otros síntomas incluyen mareo, confusión, convulsiones y alteración del nivel de conciencia.[96,97] Además de estas manifestaciones neurológicas centrales, se han descrito en la literatura hallazgos a nivel sistema nervioso periférico como alteraciones del gusto y el olfato, neuralgia y polineuropatía relacionados con la COVID-19. La hipogeusia e hiposmia se han considerado buenos predictores de diagnóstico positivo de la infección por SARS-CoV-2. [98] Un meta-análisis que incluyó un total de 4149 pacientes, encontró una prevalencia de disfunción gustativa del 57.33% y de alteración olfativa del 59.69%.[99] También se han descrito casos de síndrome de Guillain-Barré y parálisis faciales asociadas a la infección.[100,101]

3.5 Manifestaciones Cardiovasculares

Las manifestaciones cardíacas de COVID-19 incluyen arritmias cardíacas, miocarditis, pericarditis, síndrome coronario agudo, insuficiencia cardíaca, shock cardiogénico y paro cardíaco.[102] Un meta-análisis encontró una tasa de mortalidad del 10.6% en los pacientes con COVID-19 que presentaban manifestaciones cardiovasculares o hallazgos de laboratorio relacionados.[103] También cabe destacar que los pacientes con enfermedad cerebrovascular previa tienen un mayor riesgo de desarrollar COVID-19 grave. De hecho, se ha descrito una incidencia de hipertensión y enfermedades cardio y cerebrovasculares 2-3 veces mayor en los casos de COVID-19 grave.[104]

3.6 Manifestaciones Dermatológicas

Se ha observado afectación cutánea diversa en pacientes con COVID-19.[104,105] Según datos de un registro internacional que incluía 716 pacientes de 31 países, las lesiones más comunes fueron la erupción exantemática morbiliforme (22%), el eritema pernio o sabañones (18%), las lesiones tipo urticaria (16%), el eritema macular (13%), las lesiones vesiculares (11%), las papuloescamosas (9,9%) y el livedo reticularis (6,4%).[106] Este mismo estudio encontró que las lesiones de tipo pernio fueron comunes en pacientes con enfermedad leve, mientras que la livedo reticularis se presentó exclusivamente en pacientes enfermos y hospitalizados. Las lesiones acrales tipo sabañones o acro-isquémicas, han sido denominadas "dedos COVID". Estas lesiones se presentan como máculas eritemato-violáceas o purpúricas en los dedos de las manos, los codos, los dedos de los pies y la cara lateral de los pies, y se han observado con mayor frecuencia en pacientes jóvenes y niños.

3.7 Manifestaciones Oftalmológicas

Desde el origen de la pandemia, se han ido sumando progresivamente diversas alteraciones oculares en probable asociación con la infección por SARS-CoV-2. Las manifestaciones oftalmológicas descritas hasta la fecha engloban tanto afectación del segmento anterior, como del segmento posterior. No obstante, la mayoría de los signos y síntomas oftalmológicos observados durante la fase aguda de la infección afectan a la superficie ocular. De hecho, la conjuntivitis fue uno de los primeros signos identificados por los profesionales que atendían a los primeros pacientes afectados por la COVID-19.[107–109] Un meta-análisis que incluyó 19 estudios con un total de 7.300 sujetos encontró una prevalencia del 11,03% (IC del 95%: 5,71 a 17,72) de manifestaciones oculares entre los pacientes con COVID-19.[7] Los síntomas oculares más prevalentes fueron la sequedad

ocular o la sensación de cuerpo extraño (n = 138, 16,0%), el enrojecimiento ocular (n = 114, 13,3%), el lagrimeo (n = 111, 12,8%), el picor (n = 109, 12,6%), el dolor ocular (n = 83, 9,6%) y la secreción (n = 76, 8,8%), siendo la manifestación ocular más prevalente de todas las reportadas la conjuntivitis (n = 79, 88,8%). Otro estudio que incluyó un total de 2.400 pacientes, 1.200 pacientes con RT-PCR positivo para SARS-CoV-2 (grupo 1) y 1.200 con RT- PCR negativo (grupo 2), encontró que 144 (12%) pacientes del grupo 1 presentaron síntomas oculares, en comparación con 24 (2%) pacientes del grupo 2 ($p<0,001$).[110] Las manifestaciones oculares más frecuentemente observadas en el grupo 1 fueron la sensación de quemazón (6,7%, $p<0,001$), la sensación de cuerpo extraño y la irritación (7,0%, $p<0,001$), y los signos conjuntivales (2,7%, $p<0,001$). Además, la proporción de sujetos con afectación ocular aumentó en proporción a la gravedad de la COVID-19: leve (5,3%), moderada (24,6%) y grave (58,8%) ($p=0,0006$).[110] Estos síntomas también han sido reportados por los propios oftalmólogos y otros sanitarios que trabajaban en los servicios de Oftalmología de 10 hospitales de Wuhan, China, durante la primera ola.[111] La proximidad al paciente con la que los oftalmólogos realizan las exploraciones y pruebas diagnósticas es una de las principales razones por las que los oftalmólogos constituyen una de las especialidades médicas con mayor riesgo de contraer la infección por SARS-CoV-2.[112] En este sentido, Breazzano y colaboradores informaron de que el departamento de oftalmología fue una de las especialidades con mayor proporción de residentes con infección SARS-CoV-2 confirmada del total de 340 programas de residencia y 2.306 residentes con los que contaba la ciudad de Nueva York, EE.UU, entre el 2 de marzo y el 12 de abril de 2020.[113]

La mayoría de estudios coinciden en que la conjuntivitis es la manifestación oftalmológica más común en pacientes con COVID-19.[110,114–116] La frecuencia de esta manifestación ha sido objeto de múltiples estudios. [109,117–119] Sindhuja y colaboradores

informaron que el 6,29% (8 de 127) de los pacientes con COVID-19 leve incluidos presentaban congestión conjuntival,[120] cifras cercanas a las obtenidas en un estudio de mayor tamaño que incluyó 535 pacientes, donde se encontró una frecuencia del 5%.[121] En el artículo incluido en esta tesis[109] se ha estudiado la frecuencia y características clínicas de la conjuntivitis en una cohorte de pacientes ingresados con COVID-19 en el Hospital Clínico San Carlos, Madrid, durante la primera ola de la infección por SARS-CoV-2 (Marzo-Abril 2020).

En cuanto a la afectación retiniana, Marinho y colaboradores[122] describieron por primera vez la presencia de lesiones hiperreflectivas a nivel de la capa de las células ganglionares y de la capa plexiformes interna en ambos ojos mediante tomografía de coherencia óptica (OCT) , así como hemorragias retinianas y exudados algodonosos en algunos de estos pacientes. Sin embargo, los hallazgos de esta publicación han sido extensamente discutidos por otros autores, quienes consideran que los cambios encontrados en la OCT son compatibles con la configuración de los vasos normales de la retina interna.[123–125]

La tomografía de coherencia óptica constituye una técnica diagnóstica no invasiva que obtiene imágenes detalladas de la retina utilizando luz de baja coherencia. Esta herramienta constituye un método objetivo y reproducible para analizar las capas de la retina y detectar cambios en su grosor con un alto nivel de resolución.[126] Esta técnica se ha utilizado con éxito para evaluar los cambios en las capas de la retina en varias enfermedades oftalmológicas y neurológicas, como el glaucoma, la esclerosis múltiple y la enfermedad de Alzheimer.[127–129] Aunque los síntomas más comunes de la COVID-19 son de tipo respiratorio, como se ha mencionado previamente, las manifestaciones neurológicas como dolor de cabeza, mareos, hipogeusia e hiposmia durante el curso de la enfermedad son también frecuentes, lo que plantea el neurotropismo del virus, y por tanto, la capacidad de afectar las células de la retina y el nervio óptico, como integrantes del

sistema nervioso central (SNC). El mecanismo mediante el cual el virus SARS-CoV-2 accedería al SNC no ha sido esclarecido por el momento. Puesto que el receptor ACE-2 está presente en el tejido cerebral, se postula una posible invasión directa del virus.[130,131] Las dos posibles vías de acceso del virus al tejido nervioso son la vía hematógena, y la vía axonal retrógrada transináptica, a través del bulbo olfatorio y de ahí al resto de SNC. Se han observado diferencias en el grosor de la capa de células ganglionares (CCG) a nivel macular y de la capa de fibras nerviosas de la retina (CFNR) en pacientes recuperados de COVID-19 comparados con sujetos sanos, con resultados contradictorios.[132–134] Abrishami y colaboradores encontraron que el grosor de la CFNR peripapilar en todos los sectores fue mayor en los pacientes con antecedentes de COVID-19 comparado con sujetos sanos; sin embargo, estos hallazgos no alcanzaron significación estadística.[133] En el trabajo incluido en esta Tesis se analizó el grosor de las capas retinianas a nivel peripapilar y macular en pacientes recuperados de COVID-19, y se comparó con controles sanos históricos.[135]

En referencia a la afectación de la vascularización de la retina asociada a la infección por SARS-CoV-2, se ha descrito una microangiopatía retiniana asociada a la COVID-19.[136] Ésta se puede presentar con hemorragias retinianas, exudados algodonosos, tortuosidad vascular y dilatación venosa.[137–139] Según los resultados del estudio SERPICO-19 (ScrEening the Retina in Patients wIth COVID-19), que incluía un total de 187 pacientes, 54 pacientes con COVID-19 y 133 controles sanos, los hallazgos retinianos encontrados en pacientes con COVID-19 incluían: dilatación venosa (27.7%), tortuosidad vascular (12.9%), hemorragias retinianas (9.2%) y, exudados algodonosos (7.4%).[137] Además, este mismo estudió evaluó el diámetro medio de las arterias y venas retinianas, las cuales resultaron mayores en los pacientes con COVID-19 en comparación con los sujetos sanos. Asimismo, se encontró que el diámetro de las venas retinianas estaba directamente

correlacionado con la gravedad de la enfermedad. Además de los hallazgos clínicos identificados en probable relación con la COVID-19, se han publicados hallazgos subclínicos mediante angiografía por tomografía de coherencia óptica (OCTA). La OCTA es una técnica novedosa y no invasiva, que genera un angiograma tridimensional de la retina, lo que permite una evaluación cualitativa y cuantitativa de los vasos sanguíneos de la retina sin necesidad de utilizar un contraste intravenoso,[140] lo que la convierte en una técnica prometedora en el estudio de las microangiopatías y fenómenos trombóticos relacionados con la COVID-19. En comparación con las evaluaciones cualitativas, las mediciones objetivas cuantitativas de la vascularización de la retina ofrecen la posibilidad de detectar de forma precoz y precisa sutiles anomalías microvasculares no detectables clínicamente. Dado que la vascularización retiniana comparte características morfológicas y fisiopatológicas con la vasculatura de otros órganos, el estudio de la microvasculatura retiniana mediante OCTA se ha utilizado para evaluar otras enfermedades sistémicas, incluidas las cardiovasculares y las infecciosas.[141–143] Por lo tanto, la evaluación de la vascularización de la retina en pacientes con COVID-19 tiene un valor considerable, especialmente en aquellos que han sufrido complicaciones vasculares asociadas a la infección, como son los eventos trombóticos.

Se ha observado una disminución de la densidad vascular retiniana en pacientes COVID-19 tanto a nivel macular como peripapilar.[144] Además, esta disminución parece correlacionarse con la gravedad de la COVID-19, encontrando una reducción de la densidad vascular mayor en aquellos pacientes que sufrieron COVID-19 más severo.[145] Estos resultados subrayan el papel de la OCTA como posible biomarcador no invasivo de la disfunción vascular temprana tras la infección por el SARS-CoV-2, así como su asociación con parámetros clínicos y de laboratorio asociados a la enfermedad. Puesto

que los eventos trombóticos son una importante complicación de la COVID-19[146–148]y, dado que la retina es un órgano relativamente accesible, parece razonable estudiar la repercusión de estos fenómenos trombóticos en la microvascularización retiniana mediante OCTA.

HIPÓTESIS Y OBJETIVOS

4 HIPÓTESIS Y OBJETIVOS

El trabajo desarrollado en la presente Tesis se centra en el estudio de la afectación oftalmológica de la COVID-19, planteando las siguientes hipótesis:

HIPÓTESIS

- El ARN de SARS-CoV-2 está presente en la lágrima y el exudado conjuntival de pacientes con COVID-19.
- La conjuntivitis es una manifestación oftalmológica de la COVID-19.
- La COVID-19 produce cambios en la retina y en la microcirculación retiniana detectables mediante tomografía de coherencia óptica y angiografía por tomografía de coherencia óptica.

Para verificar estas hipótesis, perseguimos los siguientes objetivos de investigación:

OBJETIVOS

1. Detección de ARN de SARS-CoV-2 en lágrima y exudado conjuntival en pacientes COVID-19 con y sin conjuntivitis mediante RT-PCR
2. Establecimiento de la rentabilidad diagnóstica de la realización de RT-PCR de exudado conjuntival para el diagnóstico de COVID-19
3. Estudio de las características clínicas de la conjuntivitis en pacientes COVID-19
4. Estudio de la frecuencia de la conjuntivitis asociada a la COVID-19
5. Análisis del grosor de la capa de fibras nerviosas de la retina y capa de células ganglionares en pacientes recuperados de COVID-19 y compararlo con controles históricos

6. Análisis de la densidad vascular en sujetos COVID-19 y compararla con controles sanos mediante OCTA
7. Detección de diferencias en la densidad vascular retiniana entre sujetos COVID-19 que hayan sufrido eventos trombóticos asociados y aquellos que no
8. Estudiar las diferencias en la densidad vascular retiniana entre sujetos COVID-19 en función de los parámetros clínicos y de laboratorio

COMPENDIO DE PUBLICACIONES

5 COMPENDIO DE PUBLICACIONES

Los cinco artículos presentados en esta Tesis pueden consultarse en:

https://pubmed.ncbi.nlm.nih.gov/?term=guemes-villahoz+n

- Güemes-Villahoz N et al. Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19? J Med Virol. 2021 Jan;93(1):383-388. doi: 10.1002/jmv.26219.
- Güemes-Villahoz N et al. Conjunctivitis in COVID-19 patients: frequency and clinical presentation. Graefes Arch Clin Exp Ophthalmol. 2020 Nov;258(11):2501-2507. doi: 10.1007/s00417-020-04916-0.
- Burgos-Blasco B, Güemes-Villahoz N et al. Optic nerve and macular optical coherence tomography in recovered COVID-19 patients. Eur J Ophthalmol. 2021 Mar 15:11206721211001019. doi: 10.1177/11206721211001019.
- Güemes-Villahoz N et al. Reduced macular vessel density in COVID-19 patients with and without associated thrombotic events using optical coherence tomography angiography. Graefes Arch Clin Exp Ophthalmol. 2021 Aug;259(8):2243-2249. doi: 10.1007/s00417-021-05186-0.
- Güemes-Villahoz N et al. Reduced retinal vessel density in COVID-19 patients and elevated D-dimer levels during the acute phase of the infection. Med Clin (Barc). 2021 Jan 28:S0025-7753(21)00014-2. doi: 10.1016/j.medcli.2020.12.006.

5.1 DETECTING SARS-COV-2 RNA IN CONJUNCTIVAL SECRETIONS: IS IT A VALUABLE DIAGNOSTIC METHOD OF COVID-19?

Received: 18 June 2020 | Accepted: 22 June 2020
DOI: 10.1002/jmv.26219

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19?

Noemi Güemes-Villahoz MD | Barbara Burgos-Blasco MD[1] | Ana Arribi-Vilela PhD[2] | Pedro Arriola-Villalobos MD, PhD[3] | Carla M. Rico-Luna MD[2] | Ricardo Cuiña-Sardiña MD[1] | Alberto Delgado-Iribarren PhD[2] | Julián García-Feijoó MD, PhD[3]

[1]Servicio de Oftalmología, Hospital Clínico San Carlos, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain

[2]Department of Microbiology, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IsISSC), Madrid, Spain

[3]Servicio de Oftalmología, Departamento de Inmunología, Oftalmología y ORL, Hospital Clínico San Carlos, Facultad de Medicina, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain

Correspondence
Barbara Burgos-Blasco, MD, Department of Ophthalmology, Hospital Clinico San Carlos, Calle del Prof Martín Lagos, s/n, 28040 Madrid, Spain.
Email: bburgos171@hotmail.com

Abstract

The main purpose of this study is to evaluate the presence of viral RNA of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in conjunctival swab specimen of coronavirus disease 2019 (COVID-19) patients with and without conjunctivitis to establish the diagnostic value of reverse transcription-polymerase chain reaction (RT-PCR) in each case and to describe its clinical characteristics. A cross-sectional study was conducted at the Hospital Clinico San Carlos of Madrid, Spain. Thirty-six subjects from the COVID admission unit with laboratory-confirmed SARS-CoV-2 infection were included. Conjunctival swabs were collected from 18 patients with conjunctivitis and 18 patients without conjunctivitis and RT-PCR was performed. Conjunctival swab was collected from both eyes of 36 patients (72 eyes), detecting SARS-CoV-2 RNA in conjunctival swab of two patients (5.5%). Among the 18 patients with conjunctivitis, only one of them (5.5%) showed positive results. Likewise, SARS-CoV-2 RNA was detected in one patient without conjunctivitis (5.5%). The mean age of the 36 patients was 67.9 years (range, 28-92 years) and the male-to-female ratio was 0.44 (16:20). The mean days since the onset of COVID-19 symptoms until conjunctivitis manifestation was 8 (range, 1-24 days). The mean duration of the conjunctivitis was 3 days (range, 1-7 days). SARS-CoV-2 RNA may be detected in conjunctival swabs of both patients with and without conjunctivitis. This study revealed the same rate of positive results amongst the group with and without conjunctivitis, suggesting that detecting SARS-CoV-2 in ocular fluids is not conditioned on the presence of conjunctivitis. The presence of SARS-CoV-2 RNA in ocular samples highlights the role of the eye as a possible route of transmission of the disease.

KEYWORDS
conjunctivitis, coronavirus, COVID, diagnosis, PCR

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The disease has rapidly become a global health issue since it was first originated in China in December 2019.[1]

The main clinical features of COVID-19 are upper respiratory tract symptoms, myalgias, and diarrhea, but conjunctivitis has also been described as a clinical manifestation related to SARS-CoV-2 infection.[2,3] Evidence regarding the presence of SARS-CoV-2 RNA in tears and conjunctival secretions has been reported in patients with COVID-19.[4,5]

The main purpose of this study is to assess the presence of viral RNA of SARS-CoV-2 in conjunctival swab specimens of COVID-19 patients with and without conjunctivitis to establish the diagnostic value of reverse transcription-polymerase chain reaction (RT-PCR) in each case. Our secondary objective is to describe the clinical characteristics of conjunctivitis in a group of patients with laboratory-confirmed SARS-CoV-2 infection. The vast majority of studies published to date have been carried out in China. To the best of our knowledge, this is the first study of its kind in Europe.

2 | METHODS

This cross-sectional study was conducted at the Hospital Clinico San Carlos (HCSC) of Madrid, Spain, a tertiary referral hospital located in Madrid's metropolitan area. The study was approved by the Clinical Research Ethics Committee of this institution and was carried out in accordance with the tenets of the Declaration of Helsinki (Trial Registration Number 20/336_E_COVID). Informed consent was obtained from all patients.

Hospitalized patients for COVID-19 with and without conjunctivitis were consecutively recruited. The inclusion criteria were: over 18 years of age, positive RT-PCR test from nasopharyngeal swab for SARS-CoV-2, hospitalized due to COVID-19, and ability to give verbal consent. Critically ill patients and those unwilling or unable to give verbal consent were excluded.

For this study, a notification system was implemented for all health care personnel working at the COVID unit and evaluating the patients daily. Through this system, the ophthalmology department was notified daily of any new case of conjunctivitis amongst COVID-19 hospitalized patients. Cases reported as possible conjunctivitis were evaluated by two ophthalmologists during the first 24 hours after notification, and a conjunctival swab was collected from confirmed cases that also had positive RT-PCR from a nasopharyngeal swab. Consecutively, the sample from the patient's admitted to the following room number without conjunctivitis and confirmed SARS-CoV-2 infection was collected applying the same procedure. Both the examination and the sampling were carried out by the same physicians (N.G. and B.B.), following appropriate infection control and prevention measures. Patients with conjunctivitis were followed-up until resolution.

The conjunctival swab was collected from both eyes in every patient with a sterile synthetic fiber swab (Flexible Minitip Size Nylon Flocked Swab) into the lower fornix without topical anesthesia. We used the same swab to obtain a specimen from both eyes, first collecting the sample from the healthy eye in case of unilateral conjunctivitis. Caution was taken to avoid the possibility of sample contamination. The swab was immersed into a viral transport medium (Universal Transport Media; Copan, Italy), and stored at 4°C before being tested for SARS-CoV-2. RT-PCR assays were processed at the clinical microbiology laboratory of HCSC with quantitative GeneXpert Xpert Xpress SARS-CoV-2 (Cepheid). The cycle threshold (C_t) was measured.

The patient's age, sex, chest X-ray, laboratory test results (C-reactive protein), and days since onset of COVID-19 symptoms were obtained through the review of the patients' medical records. Also, clinical disease was classified as mild, moderate, severe, or critical based on CURB-65 score, physical examination, respiratory assessment (respiratory rate, dyspnea, blood oxygen saturation, and ventilation system requirements), or organ failure. As for the ophthalmological examination, the following variables were recorded: laterality, eye redness and discharge, duration of conjunctivitis, and treatment.

The main outcome measure is the overall proportion of positive RT-PCR test from conjunctival swab amongst COVID-19 in patients with and without conjunctivitis.

Regarding statistics, the overall prevalence results from the patients who tested positive for SARS-CoV-2 in the RT-PCR test from the conjunctival swab and it will be presented as a percentage of the total number of patients. The prevalence of positive RT-PCR among patients with conjunctivitis will be presented as a percentage of the total number of patients with conjunctivitis. The prevalence of positive RT-PCR among patients without conjunctivitis will also be presented as a percentage of the total number of patients without conjunctivitis. The distribution of sex, acute pneumonia, clinical severity, and the ophthalmological examination's findings will be presented as percentages. Likewise, the distribution of the quantitative covariates (age, C-reactive protein, days since onset of COVID-19 symptoms, and duration conjunctivitis) will be depicted through the median, first and third quartiles.

3 | RESULTS

A total of 36 patients were included in the study, 18 patients (50%) with conjunctivitis and 18 patients (50%) without conjunctivitis. Both groups had laboratory-confirmed COVID-19. Of the 689 hospitalized patients, 35 patients were reported as possible conjunctivitis, though 26 of them revealed a positive RT-PCR test from nasopharyngeal swab for SARS-CoV-2. Of those, 18 patients were finally diagnosed with conjunctivitis, three patients had a subconjunctival hemorrhage, two patients had a pterygium, one patient had eye redness related to antiglaucoma eye drops, one patient had a hordeolum, and one patient had pingueculitis. The conjunctival swab was collected from both eyes of the 36 patients included (72 eyes), detecting SARS-CoV-2 RNA in a conjunctival swab of two patients (5.5%). Among the 18 patients with conjunctivitis, one of them (5.5%) showed positive results for SARS-CoV-2 in the conjunctiva. Likewise, among the 18 COVID-19 patients without signs or symptoms of conjunctivitis, SARS-CoV-2 RNA was detected in one of them (5.5%).The mean age of the 36 patients was 67.9 years (range, 28-92 years) and the male-to-female ratio was 0.44 (16:20). Seventeen patients (47%) had mild, 12 patients (33%) had moderate disease, and seven patients (19%) had severe disease. Twenty-five of the patients (69.4%) presented pneumonia (Table 1).

The main clinical characteristics found on the patients with conjunctivitis are shown in Table 2. Half of them presented unilateral conjunctivitis and the other half were bilateral. Overall, 13 patients

TABLE 1 Clinical characteristics among patients with and without conjunctivitis

	With conjunctivitis	Without conjunctivitis
Sex		
Male (%)	39	50
Female (%)	61	50
Age, y	70.3 ± 21.6	65.4 ± 18.9
Clinical severity		
Mild (%)	50	44
Moderate (%)	33	33
Severe (%)	17	22
Pneumonia (%)	67	72

(72%) presented mild eye redness and in nine patients (50%), a moderate amount of secretions was observed. The mean days since onset of COVID-19 symptoms until conjunctivitis manifestation was 8 (range, 1-24 days). None of the patients showed conjunctival petechiae, corneal infiltrates, nor membranes or pseudomembranes. None of the patients experienced a decreased vision. The mean duration of the conjunctivitis was 3 days (range, 1-7 days).

The one patient with conjunctivitis and positive SARS-CoV-2 conjunctival swab results was a 92-year-old male classified as a severe case, but without pneumonia on chest X-ray. He presented conjunctivitis symptoms 5 days after onset of COVID-19 manifestations, which were fatigue, dizziness, and confusion. The C_t value measured in this patient was 25, which means an elevated viral load. In general, C_t levels are inversely proportional to the viral load.

Table 3 depicts the clinical characteristics of patients without conjunctivitis. Conjunctival swab samples from one patient without conjunctivitis yielded positive RT-PCR results. This patient was a 90-year-old male with multiple comorbidities, severe COVID-19, and compatible bilateral pneumonia on chest X-ray. The sample was collected 6 days after the onset of COVID-19 symptoms. Likewise, the C_t value found in this patient was 25.

4 | DISCUSSION

The novel coronavirus SARS-CoV-2 is an enveloped positive-sense RNA virus that is highly transmissible and has caused a huge global outbreak.[6] Despite the primary modes of transmission of SARS-CoV-2 infection are through respiratory droplets and contact with infected objects or surfaces, other modes of transmission,

TABLE 2 Clinical characteristics and findings in COVID-19 patients with conjunctivitis

Patient	Sex	Age, y	Clinical severity	Pneumonia	Laterality	Eye redness	Discharge	Days since onset of COVID symptoms	Duration of conjunctivitis	RT-PCR conjunctival swab
1	Female	84	2	+	unilateral	1+	1+	17	2	–
2	Male	75	3	+	bilateral	1+	2+	3	3	–
3	Male	82	2	+	bilateral	1+	2+	1	7	–
4	Female	40	1	+	bilateral	1+	1+	3	3	–
5	Female	33	3	+	bilateral	3+	1+	10	5	–
6	Female	81	1	+	unilateral	2+	2+	6	2	–
7	Female	87	2	+	unilateral	1+	1+	13	3	–
8	Male	92	3	–	bilateral	1+	2+	5	3	+
9	Male	91	2	+	bilateral	1+	2+	6	3	–
10	Female	88	1	+	unilateral	1+	2+	12	1	–
11	Female	92	1	–	unilateral	1+	2+	1	3	–
12	Female	81	1	–	unilateral	1+	1+	15	3	–
13	Female	38	1	+	unilateral	1+	2+	18	1	–
14	Female	91	2	+	unilateral	1+	2+	7	2	–
15	Male	43	1	–	bilateral	1+	1+	24	3	–
16	Male	62	1	–	bilateral	2+	1+	3	3	–
17	Male	43	2	+	bilateral	3+	1+	7	4	–
18	Female	63	1	–	unilateral	2+	1+	1	1	–

Note: 1—Mild; 2—moderate; and 3—severe.
Abbreviations: COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription-polymerase chain reaction.

TABLE 3 Clinical characteristics in COVID-19 patients without conjunctivitis

Patient	Sex	Age, y	Clinical severity	Pneumonia	Days since onset of COVID symptoms	RT-PCR conjunctival swab
1	Male	50	2	+	17	–
2	Female	63	1	+	19	–
3	Female	65	3	+	18	–
4	Female	79	1	–	8	–
5	Male	69	2	+	14	–
6	Male	90	3	+	6	+
7	Female	63	2	+	2	–
8	Male	78	3	+	16	–
9	Female	75	1	+	7	–
10	Male	35	3	+	20	–
11	Male	85	1	–	11	–
12	Female	78	2	+	6	–
13	Male	60	2	–	7	–
14	Female	28	1	+	12	–
15	Female	84	1	+	9	–
16	Male	63	1	–	3	–
17	Male	30	1	–	3	–
18	Female	82	2	+	5	–

Note: 1—Mild; 2—moderate; and 3—severe.
Abbreviations: COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription-polymerase chain reaction.

such as the ocular route, should not be overlooked, as SARS-CoV-2 RNA has been detected in tears and conjunctival secretions of patients with COVID-19.[4,5]

Previous reports have demonstrated that conjunctivitis is a clinical manifestation of COVID-19. Conjunctivitis may appear along with other COVID-19 symptoms, or may be the only presenting sign and symptom of the disease.[7] The reported prevalence of conjunctivitis varies widely among the different studies published at the time of writing this report. This prevalence ranges from 0.8%, reported by Guan et al[6] in a study that included 1099 patients with laboratory-confirmed COVID-19, to 31.6% in a case series that was also carried out in China.[3]

The natural history of the conjunctivitis in patients with COVID-19 seems to be a self-limiting conjunctivitis that improves in a few days without specific treatment. We did not find in our sample short-term complications associated to it, such as the presence of corneal infiltrates, membranes, or pseudomembranes. These characteristics differ to conjunctivitis of other etiologies, which has not been previously described.

Conjunctivitis has been associated with a more severe form of COVID-19. A recent meta-analysis showed that patients with severe COVID-19 infection had, at admission to the hospital, increased incidence of conjunctivitis.[9] These findings might have relevant clinical implications to recognize conjunctivitis as a possible sign related to a severe form of the disease. Nevertheless, our study found that only 17% of the patients with conjunctivitis had severe disease and 33% mild disease.

SARS-CoV-2 RNA has been detected in ocular fluids of patients with COVID-19 both with and without conjunctivitis.[10] However, collecting tears and ocular secretions for SARS-CoV-2 detection seem to provide a limited diagnostic value.[11] A recent study evaluated tears and conjunctival samples of 30 patients with confirmed novel coronavirus pneumonia. Of those, the only one patient with conjunctivitis revealed positive RT-PCR results.[4] Another report from Hubei providence, China, found positive results for SARS-CoV-2 on RT-PCR from both conjunctival and nasopharyngeal swabs of two patients with conjunctivitis.[2] In light of these results, it was initially suggested that the diagnostic value of the test might be greater in patients with conjunctivitis than in those without it. However, a more recent study that included 121 patients revealed that only one patient with conjunctivitis and two patients without conjunctivitis yielded positive RT-PCR results on conjunctival swab.[10] The proportion with positive results for conjunctival SARS-CoV-2 detection was 2.5% (3/121). Thus, the presence of SARS-CoV-2 RNA appears to be independent of the presence or absence of conjunctivitis associated with COVID-19. Our study included the same number of patients with and without conjunctivitis and laboratory-confirmed SARS-CoV-2 infection. Overall, our study revealed a proportion of

positive RT-PCR from conjunctival specimen of 5.5% (2/36), showing the same proportion of positive results among the conjunctivitis group and the group without conjunctivitis.

On the other hand, the two patients of our sample who tested positive for SARS-CoV-2 in conjunctival specimen were elderly people with severe forms of the disease. Likewise, the study previously mentioned by Zhou et al[10] found that two out of the three patients, that showed positive results in conjunctival swab, were classified as severe or critical cases. This brings out the possibility of detection SARS-CoV-2 in ocular secretions may be more likely in patients with severe disease. Because conjunctivitis has been associated with a more severe form of the disease, we could hypothesize that detecting SARS-CoV-2 in patients with conjunctivitis may be dependent of the severity of the disease, since both parameters appear to be interrelated.

Moreover, several studies have now established that the hyperinflammatory response induced by SARS-CoV-2 is a major cause of disease severity.[12] Thus, conjunctivitis in patients with COVID-19 could represent an inflammatory response of the disease, manifested by inflammation of the conjunctiva. The extent of the contribution of inflammation and the potential mechanisms responsible for this are still poorly understood.

PCR of nasopharyngeal specimen has demonstrated to be an effective method with overall high sensitivity and specificity for diagnosing novel coronavirus SARS-CoV-2. However, PCR essay of tears and conjunctival secretions appear to have a fairly low potential of detecting the virus, although this low positive rate of SARS-CoV-2 does not exclude the possibility of transmission of the infection through the ocular surface. Seah et al[13] evaluated the possibility of transmission through tears by assessing for the presence of SARS-CoV- 2 with viral isolation and RT-PCR analysis. A total of 64 samples were obtained during a 3 weeks period since the onset of symptoms. All samples showed negative results for SARS-CoV-2 on viral isolation and RT-PCR, suggesting that the risk of SARS-CoV-2 transmission through tears is low.

The timing of sample collection has been proposed as a factor to be considered when detecting the virus in ocular fluids. The mean days since onset of COVID-19 symptoms until sample collection were 10 days (range, 2-19). Standardized approaches for sample collection may yield more robust data about the persistence of the virus in the eye. Since most of the tears are drained into the inferior meatus of the nasal cavity, it may be possible that the virus rapidly passes from the eye surface to the respiratory system. Thus, SARS-CoV-2 would be present in the ocular surface for a limited time frame. Nevertheless, a case report by Chen et al[5] detected viral RNA in a patient with conjunctivitis for at least 5 days with the C_t values gradually increasing. Furthermore, a case report from Italy collected ocular swabs almost daily from a 65-year-old woman with conjunctivitis, detecting SARS-CoV-2 RNA for 18 consecutive days (from days 3 to 21 of the disease), and then 5 days after it became undetectable, the virus was detected again in the ocular swab sample collected at day 27.[14] These findings suggested sustained virus replication in the conjunctiva.

Hand-eye contact has been related with conjunctival congestion in patients with COVID-19. A study in 535 cases with COVID-19 found that hand-eye contact was independently correlated with conjunctival congestion.[15] Among the 27 cases with conjunctival congestion, 19 (70.4%) had a history of hand-eye contact, suggesting that frequent hand-eye contact may be a relevant risk factor for conjunctival congestion in patients with COVID-19, rather than the virus itself. The patient from our series with positive RT-PCR did not recall it, although most of the times inadvertently occurs.

This study had several limitations. First, this study includes a relatively small sample. Second, the sample was collected at different times of the disease in the different groups, which could affect the homogeneity of the results. Moreover, RT-PCR is a diagnostic test that does not possesses 100% sensitivity, so a negative test may represent a false negative result and do not rule out the presence of SARS-CoV-2. Both collecting different samples from each eye and collecting different samples over time may improve the sensitivity of the test. However, the saturation experienced by the health care system during this critical pandemic situation associated restrictions on access to patients, as well as limited resources for processing samples. Therefore, we were only able to collect one sample for both eyes from each patient. It would have been interesting to collect consecutive conjunctival specimens from those two patients who showed positive results to better understand the viral dynamics and quantify the C_t throughout the disease process.

In conclusion, SARS-CoV-2 RNA may be detected in tears and conjunctival swabs of both patients with and without conjunctivitis. Our study revealed the same rate with positive results amongst the group with and without conjunctivitis, suggesting that detecting SARS-CoV-2 in ocular fluids may not conditioned by the presence of conjunctivitis. Further studies are required to assess the risk of SARS-CoV-2 transmission through ocular secretions and the diagnostic value of RT-PCR in patients with and without conjunctivitis.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Barbara Burgos-Blasco http://orcid.org/0000-0003-2178-6164

REFERENCES

1. World Health Organization. *Pneumonia of unknown cause—China*. 2020.
2. Wu P, Duan F, Luo C, et al. Characteristics of ocular findings of patients with coronavirus disease 2019 (COVID-19) in Hubei Province, China. *JAMA Ophthalmol*. 2020;138:575-578. https://doi.org/10.1001/jamaophthalmol.2020.1291
3. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497-506. https://doi.org/10.1016/S0140-6736(20)30183-5
4. Xia J, Tong J, Liu M, Shen Y, Guo D. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *J Med Virol*. 2020;92:589-594. https://doi.org/10.1002/jmv.25725
5. Chen L, Liu M, Zhang Z, et al. Ocular manifestations of a hospitalised patient with confirmed 2019 novel coronavirus disease. *Br J Ophthalmol*. 2020;104:748-751. https://doi.org/10.1136/bjophthalmol-2020-316304

6. Khailany RA, Safdar M, Ozaslan M. Genomic characterization of a novel SARS-CoV-2. *Gene Rep*. 2020;19:100682. https://doi.org/10.1016/j.genrep.2020.100682
7. Scalinci SZ, Trovato Battagliola E. Conjunctivitis can be the only presenting sign and symptom of COVID-19. *IDCases*. 2020;20:e00774. https://doi.org/10.1016/j.idcr.2020.e00774
8. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708-1720. https://doi.org/10.1056/nejmoa2002032
9. Loffredo L, Pacella F, Pacella E, Tiscione G, Oliva A, Violi F. Conjunctivitis and COVID-19: a meta-analysis. *J Med Virol*. 2020. https://doi.org/10.1002/jmv.25938
10. Zhou Y, Duan C, Zeng Y, et al. Ocular findings and proportion with conjunctival SARS-COV-2 in COVID-19 patients. *Ophthalmology*. 2020;127:982-983. https://doi.org/10.1016/j.ophtha.2020.04.028
11. Ulhaq ZS, Soraya GV. The prevalence of ophthalmic manifestations in COVID-19 and the diagnostic value of ocular tissue/fluid. *Graefe's Arch Clin Exp Ophthalmol*. 2020;258:1351-1352. https://doi.org/10.1007/s00417-020-04695-8
12. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol*. 2020;20:355-362. https://doi.org/10.1038/s41577-020-0331-4
13. Seah IYJ, Anderson DE, Kang AEZ, et al. Assessing viral shedding and infectivity of tears in coronavirus disease 2019 (COVID-19) patients. *Ophthalmology*. 2020;127:977-979. https://doi.org/10.1016/j.ophtha.2020.03.026
14. Colavita F, Lapa D, Carletti F, et al. SARS-CoV-2 isolation from ocular secretions of a patient with COVID-19 in Italy with prolonged viral RNA detection. *Ann Intern Med*. 2020. https://doi.org/10.7326/M20-1176
15. Chen L, Deng C, Chen X, et al. Ocular manifestations and clinical characteristics of 535 cases of COVID-19 in Wuhan, China: a cross-sectional study. *Acta Ophthalmol*. 2020. https://doi.org/10.1111/aos.14472

How to cite this article: Güemes-Villahoz N, Burgos-Blasco B, Vilela AA, et al. Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19? *J Med Virol*. 2020;1–6. https://doi.org/10.1002/jmv.26219

5.2 CONJUNCTIVITIS IN COVID-19 PATIENTS: FREQUENCY AND CLINICAL PRESENTATION

Graefe's Archive for Clinical and Experimental Ophthalmology
https://doi.org/10.1007/s00417-020-04916-0

INFLAMMATORY DISORDERS

Conjunctivitis in COVID-19 patients: frequency and clinical presentation

Noemi Güemes-Villahoz[1] · Barbara Burgos-Blasco[1] · Julián García-Feijoó[2] · Federico Sáenz-Francés[2] · Pedro Arriola-Villalobos[2] · Jose María Martinez-de-la-Casa[2] · Jose Manuel Benítez-del-Castillo[2] · María Herrera de la Muela[3]

Received: 1 June 2020 / Revised: 20 July 2020 / Accepted: 25 August 2020

Abstract

Purpose The purpose of this study was to evaluate the frequency and clinical presentation of conjunctivitis in hospitalized patients with COVID-19.

Methods A cross-sectional study was conducted at the Hospital Clinico San Carlos of Madrid, Spain. A total of 301 subjects from the COVID admission unit with laboratory-confirmed SARS-CoV-2 infection were included. The presence and clinical characteristics of conjunctivitis were evaluated. Laboratory, radiological, and clinical results in patients with and without conjunctivitis stratified by sex were analyzed.

Results Of the 301 subjects included, 180 patients (59.8%) were male and the median age was 72 years (IQ 59–82). Overall, 35 patients (11.6%) were diagnosed with acute conjunctivitis. We found no relationship between the COVID-19 severity score and the presence of conjunctivitis ($P = 0.17$). However, conjunctivitis was more frequent in males with moderate clinical severity and in women classified as clinically mild. The natural history of the disease seems to be a rapid self-limited conjunctivitis that improves without treatment and does not affect visual acuity nor associate short-term complications.

Conclusions Approximately, 1 out of 10 hospitalized non-critical COVID-19 patients presents conjunctivitis during the disease. Compared with other viral conjunctivitis, we found distinctive clinical findings that could guide defining and differentiating conjunctivitis in COVID-19 patients.

Trial registration number 20/336_E_COVID

Keywords Conjunctivitis · COVID-19 · SARS-CoV-2 · Ocular · Coronavirus

Introduction

A novel coronavirus (CoV) named severe acute respiratory syndrome coronavirus–2 (SARS-CoV-2) emerged from China in December 2019. This virus causes the coronavirus disease 2019 (COVID-19), which is having an extraordinary impact worldwide [1, 2].

The references in the medical literature regarding the ocular manifestations of this emerging disease are scarce so far and, despite the fact that ocular involvement is not well defined yet, some case reports have highlighted the presence of conjunctivitis [3].

Existing data suggests that conjunctivitis is not a common manifestation associated with COVID-19 [4]. Nonetheless, characterizing conjunctival inflammation in this scenario could be of paramount importance in case it proves to be prevalent as it may be a frequent cause for seeking medical attention in patients possibly infected with SARS-CoV-2 [5].

The vast majority of studies published to date have been carried out in China. To the best of our knowledge, this is the

✉ Barbara Burgos-Blasco
bburgos171@hotmail.com

[1] Department of Ophthalmology, Hospital Clínico San Carlos, Calle del Prof Martín Lagos, s/n, 28040 Madrid, Spain

[2] Department of Ophthalmology, Hospital Clínico San Carlos, Instituto de investigación sanitaria del Hospital Clínico San Carlos (IsISSC). IIORC, Universidad Complutense de Madrid, ISCIII (OFTARED), Madrid, Spain

[3] Obstetrics and Gynecology Department, Hospital Clínico San Carlos, Instituto de salud de la mujer. Instituto de investigación sanitaria del Hospital Clínico San Carlos (IsISSC), Madrid, Spain

Published online: 29 August 2020

Key messages

- COVID-19 has been shown to have ocular involvement, mainly conjunctivitis.
- Around 12% of patients affected by COVID-19 can present conjunctivitis symptoms associated with the disease and usually occurs early in the disease.

first study of its kind in Europe. Given the current situation of the SARS-CoV-2 pandemic, describing the clinical characteristics of conjunctivitis associated with the novel coronavirus has relevant implications in the future identification of suspected COVID-19 patients and the differential diagnosis from other forms viral conjunctivitis. The purpose of this study was to evaluate the prevalence of conjunctivitis in hospitalized patients with COVID-19 and to describe its clinical presentation.

Methods

This cross-sectional study was conducted at the Hospital Clinico San Carlos of Madrid, Spain, a tertiary hospital which attends patients within the Madrid metropolitan area. The study was approved by the Clinical Research Ethics Committee of this institution and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all patients.

Hospitalized patients with laboratory-confirmed SARS-CoV-2 infection were included. Based on the hospital's protocol, the general admission criteria for patients were as follows: (1) < 50 years of age without comorbidities with bilateral pneumonia, or unilateral pneumonia with respiratory failure (saturation < 96% and respiratory rate > 20); or (2) > 50 years of age or patient with comorbidity: with pneumonia, respiratory failure (saturation < 96% and respiratory rate > 20), or laboratory/clinical severity (arterial blood gas, hemogram, D-dimer, C-reactive protein, procalcitonin, lactate dehydrogenase—LDH, transaminases).

Patients were asked about symptoms of conjunctivitis (current and previous) and they underwent a basic ophthalmological examination at their bedside by two experienced ophthalmologists on a 72-h period. A total of 301 subjects from the COVID admission unit, whose clinical situation allowed us to conduct the aforementioned ophthalmological examination and interview, were systematically explored. To examine the patients, the investigators wore double gloves, a fluid-resistant gown, a full face shield, and both FFP2 and surgical masks.

The inclusion criteria were as follows: over 18 years of age, patient with positive reverse transcriptase-polymerase chain reaction (RT-PCR) test from nasopharyngeal swab for SARS-CoV-2, hospitalized due to COVID-19, and ability to give verbal consent. Those patients admitted to the intensive care unit, unable or unwilling to give verbal consent, and unable to adequately report previous eye symptoms due to general health status were excluded.

The patient's age, sex, the onset of COVID-19 symptoms, chest X-ray, and laboratory tests results were noted. Laboratory work-up included the levels of leukocytes, neutrophils, lymphocytes, C-reactive protein, ferritin, D-dimer, creatinine, and LDH, considering the blood test results that represented the greater severity prior to the date of the ophthalmological examination. Chest X-ray results were analyzed separately, since it is not uncommon to find a discrepancy between the radiological and clinical findings, especially in the early stages of the disease. Additionally, patients were classified according to their clinical severity as mild, moderate, and severe, following the CURB-65 score, physical examination, respiratory assessment (respiratory rate, dyspnea, blood oxygen saturation, ventilation system requirements), or organ failure. The main outcome measure is the overall prevalence of conjunctivitis among inpatients diagnosed with COVID-19.

The overall prevalence results from adding patients who had conjunctivitis at the time of the evaluation and those who reported having conjunctivitis prior to the examination. Prevalence will be presented as a percentage of those with conjunctivitis along with its 95% confidence interval (CI). The distribution of gender, acute pneumonia, and bilateral pneumonia depending on the outcome status (conjunctivitis or not) will be presented as percentages and the differences addressed through the chi-squared test (of the Fisher's exact test in case the frequencies be low). Differences in prevalence and clinical presentation of conjunctivitis were analyzed by sex. Likewise, the distribution of the quantitative covariates (leucocytes, neutrophils, lymphocytes, C-reactive protein, ferritin, D-dimer, creatinine, and LDH) will be depicted through the median, first, and third quartile, using the Mann-Whitney *U* test to assess their differences depending on the presence of conjunctivitis and sex.

Results

The overall study population included 301 hospitalized patients (601 eyes). Of the 483 patients admitted to the COVID unit at the time of the study, 301 patients met the inclusion and exclusion criteria. A total of 41 patients were admitted to the intensive care unit, 135 patients were unable to adequately report previous eye events due to their clinical situation, cognitive impairment, or confusional state, and 6 patients did not give consent.

Of the 301 subjects included in the study, 180 patients (59.8%) were male and the median age was 72 years (IQ 59–82; 70 years in men and 75 years in women, $P = 0.13$). Overall, 35 patients (11.6%; 95% CI: 8.48–15.84) were diagnosed with acute conjunctivitis; of those, 10 (3.3%; 95% CI: 1.8–6.1) showed ocular manifestations on the day of the visit whereas 25 (8.3%; 95% CI: 5.6–12.1) reported having conjunctivitis in the previous days, seen by the primary care doctor. Upon ophthalmological examination of the 301 patients, other ocular disorders besides conjunctivitis were observed. Specifically, 3 patients presented subconjunctival hemorrhage, 4 patients had a moderate pterygion, and 2 patients had an hordeolum, which were managed with conservative treatment. In addition, a notification system was implemented for all healthcare personnel working at the COVID unit and evaluating the patients daily. Through this system, the on-call ophthalmologist was notified immediately with any new possible case of conjunctivitis until the date of discharge, which contacted the investigators.

Of the 35 cases that presented conjunctivitis, 13 cases suffered it before admission to the hospital, 12 cases in the time interval between admission and our evaluation, and 10 cases presented conjunctivitis at the time of evaluation. The main clinical characteristics found on the latter are shown in Table 1. None of the patients showed conjunctival petechiae, corneal infiltrates nor membranes or pseudomembranes.

The most common reported symptoms among all the 35 patients that presented conjunctivitis during the disease were mucopurulent discharge (100%; 42.8% mild, 51.4% moderate, 5.7% severe), tearing (62.8%), and foreign body sensation (57.1%). None of the patients of our study reported blurry vision associated.

The median time interval between the onset of COVID-19 symptoms and the appearance of conjunctivitis was 6 days (p25-p75: 2–13). There is no statistically significant difference in the time interval from the onset of COVID symptoms to the appearance of conjunctivitis between women and men (*U* Mann-Whitney; $P = 0.56$). According to the patient's self-report, the median duration of ocular symptoms was 3 days (p25-p75: 1–3.5) with a minimum of 1 day and a maximum of 1 week.

Table 2 depicts the distribution of the covariates analyzed depending on the conjunctivitis status, the association between the presence of conjunctivitis, and clinical, laboratory, and radiological data.

11.6% of the men and 10% of the women presented conjunctivitis. Twenty-one (60%) patients with conjunctivitis were male and 160 (60.1%) patients without conjunctivitis were male. The association between conjunctivitis and sex did not reach statistical signification ($P = 0.98$).

A total of 262 (87.0%) patients suffered from acute viral pneumonia, which was bilateral in 218 cases (82.8% of the pneumonias were bilateral). Twenty-seven (10.3%) patients with pneumonia and 6 (15.3%) patients without pneumonia presented conjunctivitis. Notwithstanding, Fisher's exact test did not allow us to consider the association between pneumonia and conjunctivitis to be causal ($P = 0.40$). Likewise, the

Table 1 Clinical presentation of conjunctivitis

		Overall ($N = 35$)				Male ($N = 21$)				Female ($N = 14$)				*P* value
		No.	%	95% CI		No.	%	95% CI		No.	%	95% CI		
Conjunctivitis	Unilateral	19	54.2	37.1	70.5	10	47.7	26.7	69.3	9	64.2	35.7	85.3	0.49*
	Bilateral	16	45.7	29.5	62.9	11	52.3	30.6	73.2	5	35.7	14.6	64.3	
Conjunctival hyperemia	Mild	28	80.0	62.6	90.5	17	80.9	57.1	93.1	11	78.5	48.1	93.5	0.99*
	Moderate/severe	7	20.0	9.4	37.4	4	19.0	6.8	42.8	3	21.4	6.4	51.8	
Mucopurulent discharge	Mild	14	42.4	26.2	60.4	7	35.0	16.4	59.5	7	53.8	24.8	80.4	0.20*
	Moderate	18	54.5	36.8	71.2	13	65.0	40.4	83.5	5	38.4	14.6	69.4	
	Severe	1	3.0	0.3	20.3	0	0.0	0.0	0.0	1	7.6	0.7	46.8	
Tearing	Yes	15	42.8	27.0	60.2	9	42.8	22.6	65.8	6	42.8	18.2	71.5	0.99*
	No	20	57.1	39.7	72.9	12	57.1	34.1	77.3	8	57.1	28.4	81.7	
Foreign body sensation	Yes	12	34.2	20.0	52.0	8	38.1	19.0	61.6	4	28.5	9.6	60.1	0.72*
	No	23	65.7	47.9	79.9	13	61.9	38.3	80.9	10	71.4	39.8	90.4	

*Fisher's exact test

Table 2 Clinical characteristics of patients with and without conjunctivitis

Measure	All ($n=301$)	Conjunctivitis ($n=35$, 11.6%)	No conjunctivitis ($n=266$, 88.4%)	*P* value	
Age, median (p25-p75)	72 (59–82)	75 (54–85)	71 (59–81)	0.38	**
Male, no. (%)	180 (59.8%)	21 (60%)	160 (60.1%)	0.98	*
Female, no. (%)	121 (40.2%)	14 (40%)	106 (39.8%)	0.98	
Pneumonia, no. (%)	262 (87.0%)	29 (82.8%)	233 (87.5%)	0.42	***
Bilateral pneumonia, no. (%)	218 (82.8%)	26 (89.6%)	192 (82.05%)	0.43	***
Leucocytes, median (p25-p75)	7 (5–9.5)	7.7 (4.9–10.4)	7 (5–9.4)	0.56	**
Neutrophils, median (p25-p75)	5.7 (3.7–7.9)	6.4 (3.4–8.2)	3.7 (5.5–7.9)	0.55	**
Lymphocytes, median (p25-p75)	0.6 (0.4–1)	0.5 (0.3–0.9)	0.6 (0.4–1)	0.32	**
CRP, median (p25-p75)	7.22 (2.2–15.2)	6.75 (1.8–14.9)	7.25 (2.3–15.7)	0.68	**
Ferritin, median (p25-p75)	589.6 (287.3–1125.1)	548.3 (224.6–948.8)	591.8 (29.8–1150.8)	0.14	**
D-dimer, median (p25-p75)	1075 (606–2146)	1126 (577–2254)	1073.5 (622–2146)	0.89	**
Creatine, median (p25-p75)	0.81 (0.62–1.13)	0.88 (0.66–1.37)	0.805 (0.6–1.1)	0.29	**
LDH, median (p25-p75)	633 (482–8269	613 (463–886)	635.5 (482–825)	0.94	**

*Chi squared

**U Mann-Whitney

***Fisher's exact test

LDH, lactate dehydrogenase; *CRP*, C-reactive protein

association between the laterality of the pneumonic process was not significantly associated with the presence of conjunctivitis ($P=0.18$).

Table 3 illustrates the laboratory, radiological, and clinical results in patients with and without conjunctivitis stratified by sex.

Table 3 Laboratory, radiological, and clinical results in patients with and without conjunctivitis stratified by sex

	Conjunctivitis							**No conjunctivitis**						
	Male			**Female**			***P***	**Male**			**Female**			***P* value**
Variables	**p50**	**p25**	**p75**	**p50**	**p25**	**p75**		**p50**	**p25**	**p75**	**p50**	**p25**	**p75**	
Leucocytes	9	6.1	10.5	6	4	9	0.03*	7.1	5	9.45	6.6	4.9	9.1	0.58*
Neutrophils	7.4	5.2	9.1	4	3	8	0.04*	5.9	3.7	8	5.3	3.4	7.9	0.20*
Lymphocytes	0.5	0.3	0.8	1	0	1	0.68*	0.6	0.4	0.8	0.7	0.4	1.1	0.01*
PCR	9.64	2.32	14.9	4	2	12	0.34*	9.1	3.4	17.25	5.32	1.13	11.2	0.001*
Ferritin	654.2	313.9	1035.1	268	103	734	0.04*	859.6	389.5	1353.6	374.5	201.1	724.4	<0.001*
D-dimer	1700	618	4513	675	380	1127	0.01*	1088.5	663.5	2117.5	1008	512	2232	0.29*
Creatinine	1.07	0.82	2	1	1	1	0.03*	0.8	0.6	1.19	0.69	0.54	0.93	<0.001*
LDH	622	485	886	591	408	699	0.59*	635.5	491.5	794.5	640	466	871	0.62*
Variable	**No. (%)**			**No. (%)**				**No. (%)**			**No. (%)**			
Pneumonia	17 (80.9%)			12 (85.7%)			0.99***	138 (86.2%)			95 (89.6%)			0.41**
Pneumonia bilat	16 (94.1%)			10 (83.3%)			0.55***	114 (82.0%)			78 (82.1%)			0.98**
Severity														
Mild	1 (4.7%)			9 (64.2%)			0.001***	68 (42.0%)			48 (45.2%)			0.17**
Moderate	12 (57.1%)			3 (21.4%)				53 (33.1%)			42 (39.6%)			
Severe	8 (38.1%)			2 (14.2%)				39 (24.3%)			16 (15.1%)			

*U Mann-Whitney

**Z-test

***Fisher's exact test

Table 4 Differences in clinical severity by gender among all patients and those with conjunctivitis

All patients (*N* = 301)			
Variable	Male	Female	*P* value
Severity:			
1	69 (38.1%)	57 (47.5%)	0.06*
2	65 (35.9%)	45 (37.5%)	
3	47 (25.9%)	18 (15%)	
Conjunctivitis (*N* = 35)			
	Male	Female	*P*
Severity:			
1	1 (4.7%)	9 (64.2%)	0.001**
2	12 (57.1%)	3 (21.4%)	
3	8 (38.1%)	2 (14.2%)	

*Chi-squared test

**Fisher's exact test

Among the 301 patients, 41.8% were classified as mild, 36.5% cases classified as moderate, and 21.5% classified as severe disease. The biochemical profiles in men and women also showed differences between them. According to the chi-squared test, there is not a relationship between the COVID-19 severity score and the presence of conjunctivitis ($P = 0.17$). However, in the analysis of conjunctivitis patients by clinical severity, there were statistically significant differences by sex (Table 4). Conjunctivitis was more frequent in males with moderate clinical severity and in women classified as clinically mild.

Discussion

Coronavirus disease 2019 (COVID-19) has shown several clinical manifestations at respiratory, gastrointestinal, and neurological levels, among others [2, 4]. Although the most frequent symptoms include respiratory symptoms, such as fever, cough, and dyspnea, the presence of conjunctivitis has also been reported [3].

The frequency of conjunctivitis in patients with COVID-19 has not been fully quantified to date, reporting very different data regarding its prevalence and incidence. A study analyzing a sample of 1099 patients hospitalized for COVID-19 disease in China found a prevalence of conjunctivitis symptoms of only 0.8% and other small series have reported a prevalence around 3% [4, 6, 7]. However, Wu P. et al. [8] found that as high as 31.6% (95% CI, 17.5–48.7) of hospitalized COVID-19 patients presented ocular signs and symptoms compatible with conjunctivitis. Our results show an 11.6% prevalence of conjunctivitis among hospitalized patients with COVID-19, differing from previous results.

Conjunctivitis as a presenting manifestation of coronavirus and the relationship between conjunctivitis and the development of serious pulmonary disease are important questions for ophthalmologists worldwide. Wu et al. [8] described in a series of 38 patients that patients with conjunctivitis were more likely to have higher white blood cell and neutrophil counts and higher levels of procalcitonin, C-reactive protein, and lactate dehydrogenase than patients without ocular symptoms, suggesting that ocular abnormalities occurred more frequently in patients with more severe COVID-19. However, this article did not compare the differences between women and men, and included critical patients, who were not included in our sample. We were unable to objectify any relationship between the presence of conjunctivitis and clinical, radiological, or laboratory severity in our sample of 301 cases. Furthermore, it is interesting to highlight that out of a hospitalized married couple who slept in the same room at home and had equal clinical severity, only the woman presented with conjunctivitis. This suggests that perhaps the appearance of conjunctivitis could depend on the host's characteristics or the inoculation mechanism.

Studies suggest that there are many differences between men and women in the immune response to SARS-CoV-19, affecting more men than women [9, 10]. Despite our study showed no difference in the clinical presentation of conjunctivitis in male and female, we found that conjunctivitis was more frequent in males with moderate COVID-19 and women with mild disease. We believe this is related to males having a more severe biochemical COVID-19 profile than females.

The clinical characteristics found in conjunctivitis associated with SARS-CoV-2 infection showed common aspects with other viral conjunctivitis, such as follicular reaction, and conjunctival hyperemia and discharge. However, we found distinctive clinical findings among our patients that could guide defining conjunctivitis in COVID-19 patients.

The differential diagnosis of SARS-CoV-2 conjunctivitis includes other viral conjunctivitis such as adenoviral conjunctivitis, based on our findings (Table 5). 54.29% of conjunctivitis were completely unilateral, unlike adenoviral conjunctivitis where there is a greater tendency to bilateralization [11]. However, bilateral conjunctivitis in COVID-19 has also been described [3].

The degree of conjunctival hyperemia was mild or very mild and the presence of follicular reaction has also been reported by other authors [3]. It was striking to find the absence of petechiae and subconjunctival hemorrhages in our sample, despite the fact that different articles reported the vascular and thrombotic complications associated with the virus [12, 13]. Also, we did not find any associated complications such as corneal infiltrates and membranes or pseudomembranes, which have not been reported in the literature so far.

The natural history of the disease seems to be a rapid self-limited conjunctivitis that improves without specific treatment. On the other hand, adenoviral conjunctivitis tends to

Table 5 Differential diagnosis between conjunctivitis in COVID-19 patients and adenoviral conjunctivitis

	Conjunctivitis in COVID-19	Adenoviral conjunctivitis
Onset	Abrupt	Abrupt
Unilateral/bilateral	Unilateral ++ Bilateral +	Unilateral or bilateral (often sequentially bilateral)
Conjunctival injection	Mild or very mild	Varies in severity
Follicular reaction	+	+
Chemosis	±	±
Eyelid swelling and erythema	–	±
Conjunctival petechiae /subconjunctival hemorrhage	–	±
Discharge	+	++
Epithelial punctate keratitis	–	±
Corneal infiltrates	–	±
Membrane/pseudomembrane formation	–	±
Concurrent upper respiratory tract infection	±	±
Symptoms	Very mild-mild	Mild-severe
Natural history	Self-limited within 2–4 days	Self-limited within 5–14 days (could get worse during the first days)
Potential sequelae	Not Known	Severe cases: conjunctival scarring, symblepharon, subepithelial corneal infiltrates, decreased vision

worsen during the first days and could last more than 14 days. The onset of conjunctivitis signs and symptoms with respect to the onset of respiratory symptoms was variable (median of 3 days). Previous reports suggest that SARS-CoV-2 can cause conjunctivitis, either as an early sign of infection or during hospitalization for severe COVID-19 [8, 14].

The fact that none of the patients reported associated blurred vision nor shown relevant epithelial corneal keratitis agrees with the case series of Wu et al. [8] However, there is a recent case report that described keratoconjunctivitis as the initial medical presentation of a patient with COVID-19 [15].

Limitations of this study include the absence of detailed ocular examinations (dilated fundus exam) to exclude intraocular disease owing to the logistical challenges of managing these patients at this critical healthcare resource situation. Since this a cross-sectional study and as such, patients that had conjunctivitis prior to admission were not evaluated by the investigators, but they were evaluated by primary care physicians.

The demonstration of the direct association between conjunctivitis and SARS-CoV-2 infection in the absence of diagnostic confirmation with real-time polymerase chain reaction (RT-PCR) of tears and conjunctival secretions is difficult to prove. However, based on previous results, the extremely low positive rate of SARS-CoV-2 RNA test by RT-PCR in tears and conjunctival secretions from patients with laboratory-confirmed SARS-CoV-2 implies that negative test results could be false negative, not excluding the presence of the virus [8, 16]. For this reason and due to the limited resources and restrictive measures of access to patients with COVID-19, RT-PCR from tears and conjunctival specimen was not tested. Based on our findings, we estimate that the actual prevalence could be underestimated, partly because many mild or very mild cases may have gone unnoticed by both healthcare personnel and the patients themselves. The exclusion of patients with cognitive impairment or confusional syndrome suggests there could also imply a shift in the actual prevalence. Since this is a tertiary hospital in downtown Madrid that covers a health area with an aging population, a high number of patients had to be excluded due to cognitive impairment, confusional state, and critical conditions, in order to obtain more reliable data. These excluded patients showed no difference in clinical characteristics compared with the included sample.

This is the first study that describes the clinical characteristics of conjunctivitis in a large sample of patients with COVID-19. A recent study carried out in China reported that the prevalence of conjunctival congestion in COVID-19 patients was 5% [17]. However, out of the 535 patients included, only 343 patients (64.1%) had laboratory-confirmed SARS-CoV-2 infection from nasopharyngeal swabs. Our study includes a total of 301 patients, all of them with the laboratory-confirmed diagnosis. Moreover, the above-mentioned article is a retrospective study where the patients were not evaluated by an ophthalmologist, and the patient's data were obtained from patients' electronic medical records and an electronic questionnaire completed by patients on a smartphone. Furthermore, data about ocular manifestations were obtained by ophthalmologists via telephone, so results are determined by patient subjectivity and ophthalmologist's interpretation. This makes our study the most comprehensive and extensive of its category.

COVID-19 has spread rapidly since it was first identified in Wuhan and has been shown to have ocular involvement, mainly conjunctivitis. We found a prevalence of conjunctivitis in our sample of 11.6%, which allows us to infer that approximately 1 in 10 patients affected by COVID-19 can present conjunctivitis symptoms associated with the disease.

Our observations can help ophthalmologists and other physicians to identify possible COVID-19 patients presenting with red eye or discharge as main complain for seeking care, especially in women who could present conjunctivitis earlier in the disease. However, at this point in the COVID-19 pandemic, it is reasonable that practically any patient seen by a medical practitioner is considered suspected of SARS-CoV-2 infection, regardless of presenting signs or symptoms of conjunctivitis.

A better understanding of the ocular manifestations of the virus will assist in early identification of SARS-CoV-2-infected cases, prioritizing diagnostic testing in patients with clinical findings compatible with conjunctivitis associated with COVID-19.

Data availability Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Compliance with ethical standards

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Huang Y, Tu M, Wang S et al (2020) Clinical characteristics of laboratory confirmed positive cases of SARS-CoV-2 infection in Wuhan, China: a retrospective single center analysis. Travel Med Infect Dis:101606. https://doi.org/10.1016/j.tmaid.2020.101606
2. Huang C, Wang Y, Li X et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395(10223):497–506. https://doi.org/10.1016/S0140-6736(20)30183-5
3. Chen L, Liu M, Zhang Z et al (2020) Ocular manifestations of a hospitalised patient with confirmed 2019 novel coronavirus disease. Br J Ophthalmol:1–4. https://doi.org/10.1136/bjophthalmol-2020-316304
4. Guan W, Ni Z, Hu Y et al (2020) Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med:1–13. https://doi.org/10.1056/nejmoa2002032
5. Ramirez DA, Porco TC, Lietman TM, Keenan JD (2017) Epidemiology of conjunctivitis in US emergency departments. JAMA Ophthalmol 135(10):1119–1121. https://doi.org/10.1001/jamaophthalmol.2017.3319
6. Xia J, Tong J, Liu M, Shen Y, Guo D (2020) Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J Med Virol:1–6. https://doi.org/10.1002/jmv.25725
7. Zhang X, Chen X, Chen L et al (2020) The evidence of SARS-CoV-2 infection on ocular surface. Ocul Surf. https://doi.org/10.1016/j.jtos.2020.03.010
8. Wu P, Duan F, Luo C et al (2020) Characteristics of ocular findings of patients with coronavirus disease 2019 (COVID-19) in Hubei Province, China. JAMA Ophthalmol:1–8. https://doi.org/10.1001/jamaophthalmol.2020.1291
9. Conti P, Younes A (2020) Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection. J Biol Regul Homeost Agents 34(2). https://doi.org/10.23812/Editorial-Conti-3
10. Yi Y, Lagniton PNP, Ye S, Li E, Xu R-H (2020) COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci 16(10):1753–1766. https://doi.org/10.7150/ijbs.45134
11. Yeu E, Hauswirth S (2020) A review of the differential diagnosis of acute infectious conjunctivitis: implications for treatment and management. Clin Ophthalmol 14:805–813. https://doi.org/10.2147/OPTH.S236571
12. Giannis D, Ziogas IA, Gianni P (2020) Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. J Clin Virol 127:104362. https://doi.org/10.1016/j.jcv.2020.104362
13. Xiong M, Liang X, Wei Y-D (2020) Changes in blood coagulation in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. Br J Haematol. https://doi.org/10.1111/bjh.16725
14. Hu K, Patel J, Patel BC (2020) Ophthalmic manifestations of coronavirus (COVID-19). StatPearls 9:1–9 http://www.ncbi.nlm.nih.gov/pubmed/32310553
15. Cheema M, Aghazadeh H, Nazarali S et al (2020) Keratoconjunctivitis as the initial medical presentation of the novel coronavirus disease 2019 (COVID-19): a case report. Can J Ophthalmol. https://doi.org/10.1016/j.jcjo.2020.03.003
16. Zhou Y, Duan C, Zeng Y et al (2020) Ocular findings and proportion with conjunctival SARS-COV-2 in COVID-19 patients. Ophthalmology. https://doi.org/10.1016/j.ophtha.2020.04.028
17. Chen L, Deng C, Chen X et al (2020) Ocular manifestations and clinical characteristics of 535 cases of COVID-19 in Wuhan, China: a cross-sectional study. Acta Ophthalmol. https://doi.org/10.1111/aos.14472

5.3 OPTIC NERVE AND MACULAR OPTICAL COHERENCE TOMOGRAPHY IN RECOVERED COVID-19 PATIENTS

Original research article

EJO European Journal of Ophthalmology

European Journal of Ophthalmology
2022, Vol. 32(1) 628–636

DOI: 10.1177/1120672121001019
journals.sagepub.com/home/ejo
SAGE

Optic nerve and macular optical coherence tomography in recovered COVID-19 patients

Barbara Burgos-Blasco[1], Noemi Güemes-Villahoz[1], Beatriz Vidal-Villegas[1], Jose Maria Martinez-de-la-Casa[1], Juan Donate-Lopez[1], Francisco Javier Martín-Sánchez[2], Juan Jorge González-Armengol[2], Jesus Porta-Etessam[3], Jose Luis R Martin[4] and Julian Garcia-Feijoo[5]

Abstract

Purpose: To investigate the peripapillary retinal nerve fiber layer thickness (RNFLT), macular RNFLT, ganglion cell layer (GCL), and inner plexiform layer (IPL) thickness in recovered COVID-19 patients compared to controls.

Methods: Patients previously diagnosed with COVID-19 were included, while healthy patients formed the historic control group. All patients underwent an ophthalmological examination, including macular and optic nerve optical coherence tomography. In the case group, socio-demographic data, medical history, and neurological symptoms were collected.

Results: One hundred sixty patients were included; 90 recovered COVID-19 patients and 70 controls. COVID-19 patients presented increases in global RNFLT (mean difference 4.3; CI95% 0.8 to 7.7), nasal superior (mean difference 6.9; CI95% 0.4 to 13.4), and nasal inferior (mean difference 10.2; CI95% 2.4 to 18.1) sectors of peripapillary RNFLT. Macular RNFL showed decreases in COVID-19 patients in volume (mean difference −0.05; CI95% −0.08 to −0.02), superior inner (mean difference −1.4; CI95% −2.5 to −0.4), nasal inner (mean difference −1.1; CI95% −1.8 to −0.3), and nasal outer (mean difference −4.7; CI95% −7.0 to −2.4) quadrants. COVID-19 patients presented increased GCL thickness in volume (mean difference 0.04; CI95% 0.01 to 0.07), superior outer (mean difference 2.1; CI95% 0.8 to 3.3), nasal outer (mean difference 2.5; CI95% 1.1 to 4.0), and inferior outer (mean difference 1.2; CI95% 0.1 to 2.4) quadrants. COVID-19 patients with anosmia and ageusia presented an increase in peripapillary RNFLT and macular GCL compared to patients without these symptoms.

Conclusions: SARS-CoV-2 may affect the optic nerve and cause changes in the retinal layers once the infection has resolved.

Keywords

COVID, coronavirus, optical coherence tomography, optic nerve

Date received: 22 January 2021; accepted: 14 February 2021

[1]Ophthalmology Department, Hospital Clínico San Carlos, Madrid, Spain
[2]Emergency Department, Hospital Clínico San Carlos, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdiSCC), Universidad Complutense de Madrid, Spain
[3]Neurology Department, Hospital Clínico San Carlos, Madrid, Spain
[4]Simplifying Research Institute, Madrid, Spain
[5]Ophthalmology Department, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IsISSC), IIORC, Universidad Complutense, Madrid, Spain

Corresponding author:
Barbara Burgos-Blasco, Ophthalmology Department, Hospital Clínico San Carlos, Calle del Prof Martín Lagos, Madrid 28040, Spain.
Email: bburgos171@hotmail.com

Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes the coronavirus disease 2019 (COVID-19) emerged in Wuhan, China in December 2019 and is having devastating consequences worldwide.[1]

COVID-19 has shown clinical manifestations at nearly all levels, although its major clinical finding is pneumonia, which may eventually lead to an immense respiratory distress. Ground glass opacities and consolidations are common lung computed tomography abnormalities in COVID-19 pneumonia, which express acute lung injury.[2] Common symptoms include fatigue, fever, cough, and diarrhea, as well as characteristic laboratory findings such as elevated serum levels of leucocytes, D-dimer, C-reactive protein, procalcitonin, IL-6, urea, and creatinine, among others.[3,4]

Neurologic manifestations have also been reported in a notable proportion of patients. Several studies hypothesize that the virus may penetrate the central nervous system (CNS) producing neurological complications, including anosmia, ageusia, encephalopathy, headache, ataxia, epileptic seizures, and cerebrovascular disease.[5,6]

Regarding the ocular involvement, ocular surface disorders have been described, mainly conjunctivitis which has been reported in around 10% of patients.[7] However, little is known about how it affects the retina and the optic nerve as part of the CNS.[8]

Optical coherence tomography (OCT) is a non-invasive imaging technique that obtains detailed images of the retina using low-coherence light. It is a reliable and reproducible method for measuring retinal layers and detecting changes in layer thickness with a high level of resolution.[9] This technique has been successfully used to monitor changes in the retinal layers in a number of ophthalmological and neurological diseases, such as glaucoma, multiple sclerosis, and Alzheimer disease.[10,11]

Currently there are no studies that have investigated the effect of COVID-19 on the optic nerve and the macula. The primary objective was to evaluate the peripapillary retinal nerve fiber layer thickness (RNFLT), macular RNFLT, ganglion cell layer (GCL), and inner plexiform layer (IPL) thickness in patients with COVID-19 compared to healthy controls. The secondary objective was to study the relation between macular and optic nerve findings and neurological symptoms.

Methods

Subjects and setting

This case-control study was conducted at the Hospital Clinico San Carlos, a tertiary hospital sited in Madrid (Spain). The study was approved by the hospital's Clinical Research Ethics Committee and was conducted in accordance with the Helsinki Declaration. All patients provided written informed consent.

The case group was formed by patients with COVID-19 who presented in the hospital's Emergency Department (ED) between 23 and 29 March, 2020 and successfully recovered from the infection. The inclusion criteria were: between 18 and 70 years of age; SARS-CoV-2 infection confirmed by positive reverse transcriptase–polymerase chain reaction (RT-PCR) test from nasopharyngeal swab, and written informed consent.

Patients were asked to come to the hospital for the study if inclusion criteria were met. Those patients, still presenting symptoms, on quarantine, unable to attend the hospital due to general health status, as well as those with concomitant psychiatric, neurological, or ophthalmological diseases were excluded. The latter included optic nerve head disease (including glaucoma and congenital optic nerve head abnormalities), macular disease, retinal vascular disorders, high myopia (refractive error greater than six diopters), uveitis, and history of previous ophthalmic procedures other than cataract surgery and capsulotomy.

The control group was formed by historic healthy controls recruited for a normative database in 2018. Due to the difficulty in obtaining controls and being certain of no history of virus infection (specificity and sensitivity of diagnostic tests is not 100% and a high prevalence of asymptomatic patients has been reported), it was decided to use historical controls within the same age group from previous studies that had undergone the same tests (same device and same software). Controls were between 18 and 70 years of age and without ophthalmological pathology (the same criteria that were applied to the study group). The control group was matched by age, sex, and refraction.

Patients unable or unwilling to give consent as well as those with media opacity and poor-quality images were excluded from both groups.

Ophthalmologic exam and optic nerve imaging

All patients underwent an ophthalmological examination, including slit-lamp biomicroscopy, funduscopy, and OCT. These exams were performed 4 weeks after COVID-19 diagnosis in order to fully comply mandatory isolation in the case group and from January 2018 to June 2018 in the historic controls.

The Spectralis-OCT (Heidelberg Engineering, Heidelberg, Germany) was used to obtain the structural measurements of the retina.

Macular OCT was performed using a dense macular cube protocol, where a 6 × 6 mm area on the retina was scanned. With the ETDRS macular scan, nine ETDRS macular areas are scanned (including a central 1 mm circle, and inner and outer rings measuring 3 and 6 mm in diameter), central, and average layer thickness are analyzed (Figure 1). In the macular area, RNFLT (between the inner limiting membrane and the GCL), GCL thickness (between RNFL and the IPL), and IPL thickness (between

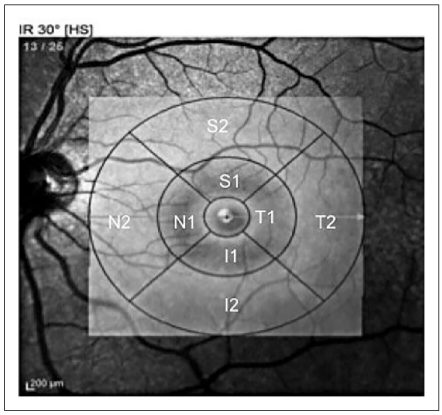

Figure 1. Macular sectors of the optical coherence tomography map.
T: temporal; S: superior; I: inferior; N: nasal; 1: inner sector; 2: outer sector.

GCL and the inner nuclear layer) were obtained using the device's software.

The optic nerve head was scanned and peripapillary RNFL thickness measurements were made using a circular scan pattern which was centered on the optic nerve. The Spectralis OCT software calculates RNFLT as a result of automated segmentation. RNFL measurements were noted globally and in the six quadrants (superior temporal, temporal, inferior temporal, superior nasal, nasal, inferior nasal).

A single experienced physician carried out all OCT examinations. Only OCT images with a signal strength level above the recommended signal strength level (>7/10) were obtained. One eye per patient, which was randomly selected, was included.

Comparison between neurological symptoms and ophthalmologic parameters in COVID-19

Socio-demographic data (age, sex, and race), medical history (hypertension, diabetes mellitus, dyslipidemia), and clinical severity at ED presentation were collected.

Clinical data was obtained from the electronic medical records, and two physicians (NGV and BBB) checked the data on the neurological symptoms (dizziness, headache, ageusia, anosmia). Electronic medical records were checked for COVID-19 symptoms and questioning on the examination day was used to confirm this.

Statistical methods

Data analysis was performed using SPSS software, version 24.00 (IBM, New Castle, NY, USA), and STATA version 15.1 (Stata Corp, College Station, TX, USA). Continuous variables are presented as mean and standard deviation (SD), while numbers and percentages are used for categorical variables. Differences in age and sex between groups were compared using the Chi^2 test and *t*-student test. Variables normality was evaluated using Kolmogorov-Smirnov test.

For the primary objective, investigation of the differences in the OCT variables between the control group and the patients with COVID-19, a *t*-student test was performed. For the secondary objective, comparison between the group of patients with and without neurological symptoms, a *t*-student test was performed. Statistical significance was established in 0.05.

Results

The study comprised a total of 160 patients; 90 of them recovered from SARS-CoV-2 infection and 70 healthy historical controls.

Of the 584 patients diagnosed with COVID-19 in the ED, 234 patients met the inclusion criteria. Of those, 7 patients died, 19 patients were still admitted to the COVID-19 unit; 13 patients were unable to return to the hospital due to their clinical situation; 12 patients still presented respiratory symptoms after discharge; 29 patients had concomitant ocular disorders, 11 patients did not give consent and 49 patients were lost to follow-up after emergency department discharge. Four patients were excluded because the OCT image did not meet the quality criteria (signal strength, segmentation error, loss of fixation, and motion artifacts).

Among the 70 control subjects included, 30 patients (43%) were male and 40 patients were female (57%), being the mean age 55.5 years (SD 14.9 years), and the mean refractive error −0.36 Dp (SD 1.53 Dp). The main clinical characteristics of the patients with resolved SARS-CoV-2 infection are shown in Table 1. Mean age of the case group was 55.5 years (SD 8.9 years), 49% of patients being male and mean refractive error −0.49 Dp (SD 1.78 Dp). Fifty-three patients (59%) presented anosmia or ageusia as COVID-19 symptoms and 55 (61%) presented headache or dizziness. No statistically significant differences in the sex, age, and refractive error between both groups were noted ($p > 0.05$).

Funduscopic examination of recovered COVID-19 patients included was unremarkable, not showing visible optic disc oedema/swelling, nor related clinical features. None of the patients included in our study reported visual loss.

Tables 2 and 3 depict the distribution and univariable analysis of the OCT parameters in the control and case group. Post-COVID-19 patients showed statistically significant increases in the global RNFLT (mean difference 4.3; CI95% 0.8 to 7.7), as well as superior nasal (mean

difference 6.9; CI95% 0.4 to 13.4) and inferior nasal (mean difference 10.2; CI95% 2.4 to 18.1) sectors of the peripapillary RNFLT (Figure 2). Macular RNFL parameters showed decreases in recovered COVID-19 patients in volume (mean difference −0.05; CI95% −0.08 to −0.02), as well as the superior inner (mean difference −1.4; CI95% −2.5 to −0.4), nasal inner (mean difference −1.1; CI95% −1.8 to −0.3), and nasal outer (mean difference −4.7; CI95% −7.0 to −2.4) quadrants (Figure 3(a)). Comparison of GCL thickness between recovered COVID-19 patients and controls showed increased thickness in the former in volume (mean difference 0.04; CI95% 0.01 to 0.07), superior outer (mean difference 2.1; CI95% 0.8 to 3.3), nasal outer (mean difference 2.5; CI95% 1.1 to 4.0) and inferior outer (mean difference 1.2; CI95% 0.1 to 2.4) quadrants (Figure 3(b)). IPL thickness did not reveal significant differences (global and sectors, all $p > 0.05$).

Table 1. Demographic and clinical characteristics of COVID-19 patients.

	N = 90
Sociodemographic data	
Age, years. Mean (SD)	55.5 (8.9)
Sex, male. No (%)	44 (48.9)
Race	
Caucasic. No (%)	60 (66.7)
Hispanic. No (%)	30 (33.3)
Medical history	
Hypertension No (%)	26 (28.9)
Diabetes mellitus No (%)	8 (8.9)
Dyslipidemia No (%)	25 (27.8)
Neurological symptoms	
Anosmia/ageusia. No (%)	53 (58.9)
Headache/dizziness. No (%)	55 (61.1)
Clinical severity	
Mild. No (%)	31 (34.4)
Moderate. No (%)	23 (25.6)
Severe. No (%)	36 (40.0)

SD: standard deviation.

Post-COVID-19 patients with anosmia and ageusia during the infection presented a significant increase in some regions of peripapillary RNFLT and macular GCL compared to COVID-19 patients who had not referred these symptoms (Table 4). There were no differences in peripapillary RNFL between controls and patients without anosmia or ageusia (global and sectors, all $p > 0.05$). The association between having headache or dizziness and the OCT layers did not reach statistical signification (global and sectors, all $p > 0.05$).

Discussion

Neurological involvement in COVID-19 has already been described and highlights the relevance of considering the neurological impact of SARS-CoV-2. In this study, the peripapillary RNFLT and the inner retina in patients with SARS-CoV-2 infection were analyzed, presenting recovered COVID-19 patients an increase in peripapillary RNFLT and macular GCC compared to controls. Patients with anosmia and ageusia also showed increased peripapillary RNFLT and macular GCL thickness compared to COVID-19 patients without these symptoms during the infection.

Neurological manifestations are described in 30–40% of COVID-19 patients, being CNS manifestations such as dizziness and headache more frequent, followed by peripheral nervous system (PNS) and skeletal muscle injury.[5] Ageusia and anosmia have also been reported as common clinical features, with a frequency ranging from 20% to 90% of patients and are considered highly specific for COVID-19.[5,12–14] In our series, prevalence of neurological manifestations (anosmia/ageusia 59%, headache/dizziness 61%) was slightly higher than in other series, perhaps due to our thoroughness on questioning about these symptoms.

Reports on ocular involvement in SARS-CoV-2 infection are scarce. Invernizzi et al.[15] assessed the presence of retinal alterations in patients with COVID-19 using fundus photographs, detecting hemorrhages (9.25%), cotton wools spots (7.4%), dilated veins (27.7%), and tortuous

Table 2. Peripapillary optical coherence tomography (OCT) results in healthy controls and COVID-19 patients.

Optic nerve OCT	COVID+ (*n* = 88)		COVID − (*n* = 70)		*p*	Mean differences	CI 95%
	Mean	SD	Mean	SD			
RNFL G (μm)	101.4	10.2	97.1	11.7	**0.015**	4.3	0.8 to 7.7
RNFL T (μm)	70.0	11.3	68.6	13.0	0.467	1.4	−2.4 to 5.2
RNFL TS (μm)	136.1	22.7	132.5	17.8	0.281	3.6	−3.0 to 10.2
RNFL TI (μm)	141.2	22.0	137.5	20.1	0.276	3.7	−3.0 to 10.4
RNFL N (μm)	79.2	13.5	76.0	16.5	0.179	3.2	−1.5 to 7.9
RNFL NS (μm)	112.0	20.4	105.1	21.2	**0.039**	6.9	0.4 to 13.4
RNFL NI (μm)	123.9	24.9	113.6	24.8	**0.011**	10.2	2.4 to 18.1

SD: standard deviation; CI: confidence interval; RNFL: retinal nerve fiber layer; G: global; T: temporal; TS: temporal-superior; TI: temporal-inferior; N: nasal; NS: nasal-superior; NI: nasal inferior.
Significant differences are shown in bold.

Table 3. Macular optical coherence tomography (OCT) in healthy controls and COVID-19 patients.

Macular OCT	COVID+ ($n=90$)		COVID − ($n=70$)		p	Mean differences	CI 95%
	Mean	SD	Mean	SD			
RNFL volume (μm^3)	0.90	0.11	0.95	0.09	**0.002**	−0.05	−0.08 to −0.02
RNFL S1 (μm)	23.8	3.5	25.3	3.3	**0.009**	−1.4	−2.5 to −0.4
RNFL S2 (μm)	36.8	6.1	38.6	5.8	0.059	−1.8	−3.7 to 0.1
RNFL N1 (μm)	20.7	2.5	21.7	2.2	**0.006**	−1.1	−1.8 to −0.3
RNFL N2 (μm)	46.5	7.8	51.2	6.5	**0.000**	−4.7	−7.0 to −2.4
RNFL I1 (μm)	25.2	3.7	26.0	3.4	0.166	−0.8	−1.9 to 0.3
RNFL I2 (μm)	39.5	5.8	41.1	5.3	0.071	−1.6	−3.4 to 0.1
RNFL T1 (μm)	17.7	1.8	17.8	1.2	0.900	−0.1	−0.5 to 0.5
RNFL T2 (μm)	19.2	1.5	19.5	1.5	0.298	−0.3	−0.7 to 0.2
GCL volume (μm^3)	1.09	0.10	1.05	0.11	**0.028**	0.04	0.01 to 0.07
GCL S1 (μm)	51.3	5.9	50.4	6.1	0.371	0.9	−1.0 to 2.7
GCL S2 (μm)	35.4	4.0	33.4	4.0	**0.001**	2.1	0.8 to 3.3
GCL N1 (μm)	50.3	6.5	49.5	5.8	0.410	0.8	−1.1 to 2.8
GCL N2 (μm)	39.0	4.0	36.5	4.9	**0.001**	2.5	1.1 to 4.0
GCL I1 (μm)	51.3	5.3	49.8	6.3	0.098	1.6	−0.3 to 3.4
GCL I2 (μm)	33.6	3.3	32.4	3.8	**0.028**	1.2	0.1 to 2.4
GCL T1 (μm)	46.3	5.6	45.8	5.2	0.622	0.4	−1.3 to 2.1
GCL T2 (μm)	35.9	4.4	35.0	3.9	0.161	0.9	−0.4 to 2.3

SD: standard deviation; CI: confidence interval; RNFL: retinal nerve fiber layer; T: temporal; S: superior; I: inferior; N: nasal; 1: inner sector; 2: outer sector; GCL: ganglion cell layer; IPL: inner plexiform layer.
Significant differences are shown in bold.

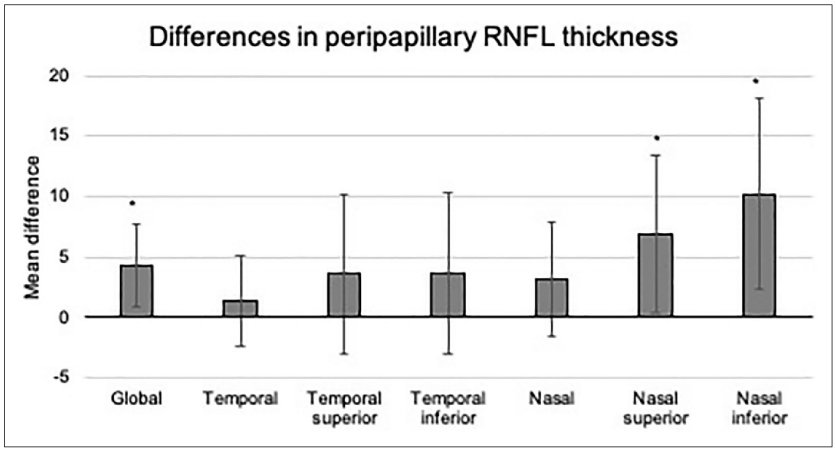

Figure 2. Differences in retinal nerve fiber layer (RNFL) thickness between COVID-19 patients and healthy controls.
RNFL thickness is measured in μm. *$p < 0.05$.

vessels (12.9%). Mean arteries diameter and mean veins diameter were higher in COVID-19 patients compared to unexposed subjects using computer-based analysis, retinal vessels dilation not always being detectable by clinical funduscopic examination. These changes could be due to inflammation, hypoxia or an increase in CO_2.

Savastano et al.[16] evaluated peripapillary RNFL and vascularization of 80 COVID-19 patients compared to 30 healthy controls. Radial peripapillary capillary plexus (RPCP) perfusion density was lower in COVID-19 patients, correlating with age, as well as treatment with lopinavir/ritonavir or antiplatelet therapy. RNFL average thickness was linearly correlated to RPCP flow index and perfusion density within post-COVID-19 group. However, no differences in RNFL average thickness were observed between COVID-19 patients and healthy controls.

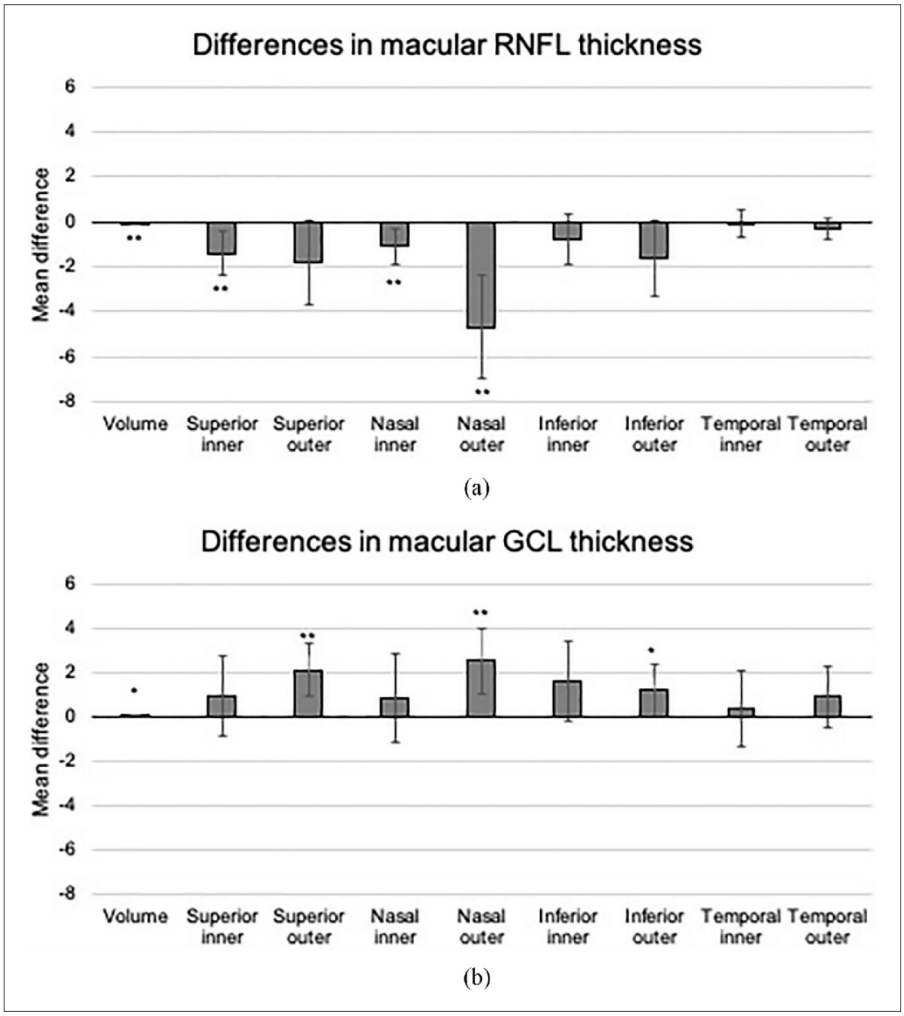

Figure 3. Macular optical coherence tomography analysis showing differences in (a) macular retinal nerve fiber layer (RNFL) thickness and (b) ganglion cell layer (GCL) thickness between COVID-19 patients and healthy controls.
Sectors are measured in μm, volumes are measured in μm^3. *$p < 0.05$. **$p < 0.01$.

Despite its immune-privileged status, the CNS can respond quickly and intensely to virus.[17] Neurotropic viral infections can cause brain parenchyma inflammation, reports of encephalitis caused by SARS-CoV-2 proving this mechanism.[18,19] Our results note increases in peripapillary RNFLT and macular GCL compared to patients without COVID-19, which could suggest an effect of the virus on the optic nerve.[20] This increase is supported by other results from our group that observed an increase in peripapillary RNFLT in recovered COVID-19 patients in comparison to examinations prior to the infection.[9] Our results contrast with those reported by Savastano et al.,[16] but, as they acknowledge, they present a low sample of healthy controls and an asymmetry between groups. In addition, only 6.25% of the subjects required admission to the intensive care unit and 8.8% required non-invasive ventilation, while 40% of the patients in our group presented a severe form of the disease. All of this could explain why differences were detected in the present study.

Table 4. Optic nerve and macular optical coherence tomography (OCT) in COVID-19 patients with anosmia or ageusia.

	Without symptoms ($n=37$)		With symptoms ($n=53$)		p	Mean differences	CI 95%
	Mean	SD	Mean	SD			
RNFL G (µm)	98.5	10.5	103.4	9.7	**0.026**	−4.9	−9.2 to −0.6
RNFL T (µm)	69.1	9.7	70.6	12.3	0.557	−1.4	−6.3 to 3.4
RNFL TS (µm)	130.4	22.4	140.1	22.3	**0.047**	−9.7	−19.2 to −0.1
RNFL TI (µm)	133.2	19.6	146.7	22.1	**0.004**	−13.5	−22.6 to −4.4
RNFL N (µm)	78.0	14.3	80.0	13.0	0.495	−2	−7.8 to 3.8
RNFL NS (µm)	108.2	22.4	114.6	18.6	0.145	−6.4	−15.1 to 2.3
RNFL NI (µm)	122.1	28.3	125.0	22.5	0.594	−2.9	−13.7 to 7.9
M RNFL volume (µm^3)	0.91	0.11	0.89	0.10	0.448	0.02	−0.03 to 0.06
M RNFL S1 (µm)	24.5	4.0	23.4	3.0	0.132	1.1	−0.3 to 2.6
M RNFL S2 (µm)	36.8	6.7	36.8	5.7	0.979	0.1	−2.6 to 2.7
M RNFL N1 (µm)	21.4	2.9	20.2	2.0	**0.03**	1.2	0.1 to 2.3
M RNFL N2 (µm)	47.9	8.4	45.4	7.2	0.136	2.5	−0.8 to 5.8
M RNFL I1 (µm)	26.2	4.0	24.5	3.3	**0.023**	1.8	0.3 to 3.3
M RNFL I2 (µm)	38.5	5.4	40.2	6.0	0.174	−1.7	−4.1 to 0.8
M RNFL T1 (µm)	18.1	2.3	17.5	1.2	0.110	0.7	−0.1 to 0.4
M RNFL T2 (µm)	19.4	1.4	19.1	1.5	0.309	0.3	−0.3 to 1.0
GCL volume (µm^3)	1.07	0.10	1.09	0.10	0.400	−0.02	−0.06 to 0.02
GCL S1 (µm)	51.1	6.7	51.4	5.3	0.798	−0.3	−2.9 to 2.2
GCL S2 (µm)	35.3	3.9	35.5	4.1	0.772	−0.2	−2.0 to 1.5
GCL N1 (µm)	50.9	5.9	49.8	6.8	0.424	1.1	−1.6 to 3.9
GCL N2 (µm)	38.0	3.9	39.7	3.9	**0.045**	−1.7	−3.4 to −0.1
GCL I1 (µm)	51.5	4.7	51.2	5.6	0.796	0.3	−2.0 to 2.6
GCL I12 (µm)	32.5	3.1	34.5	3.2	**0.004**	−2.0	−3.3 to −0.6
GCL T1 (µm)	45.8	5.8	46.6	5.4	0.529	−0.8	−3.1 to 1.6
GCL T2 (µm)	35.5	4.9	36.2	4.1	0.455	−0.7	−2.6 to 1.2

SD: standard deviation; CI: confidence interval; RNFL: retinal nerve fiber layer; M: macular; T: temporal; S: superior; I: inferior; N: nasal; 1: inner sector; 2: outer sector; GCL: ganglion cell layer; IPL: inner plexiform layer.
Significant differences are shown in bold.

In multiple neurodegenerative diseases involvement of the inner retinal layers using OCT has been reported. In Parkinson, the Braak hypothesis for its etiology is based on a neurotropic virus that invades the nervous system. Interestingly, the preclinical phase of Parkinson may present olfactory and gastrointestinal symptoms, similarly to COVID-19.[21] In OCT of Parkinson patients, peripapillary RNFLT, GCL, IPL, and retinal thickness are thinner as a result of nervous damage.[22–25] Hence, the increases observed in our series could be due to acute damage, which could turn into atrophy in the long-term. Moreover, multiple sclerosis might be triggered by an infectious agent, a virus being the most likely cause. Animal models describe that the best method to induce neuroinflammation is intracranial inoculation, leading to optic nerve inflammation.[26] Optic neuritis secondary to multiple sclerosis are commonly posterior, but anterior optic neuritis in other diseases typically cause increases in peripapillary RNFLT and clinically significant oedema.

As with other neurotropic viruses, SARS-COV-2 may invade the CNS through various routes, including the hematogenous or retrograde neuronal route. Neurological manifestations may be due to the CNS invasion of the virus, similar to other CoV.[27,28] On the other hand, smell impairment is a typical feature of SARS-CoV-2 and a possible neural pathway given by the olfactory nerve has been found in models.[29] Recent reports have demonstrated virus RNA in the human retina, the optic nerve could thus be affected through a transynaptic retrograde pathway from the olfactory bulb.[30,31] Through its binding to angiotensin-converting enzyme 2 (ACE2) receptors, the virus may access the PNS and travel transneuronally to the CNS, similar to other neurotropic viruses.[17] In this sense, anosmia due to virus damage to the olfactory pathways could be justified by this mechanism. The brain also has a high expression of ACE2 receptors, which supports the high penetration of the virus into the CNS.[32]

The changes observed when post-COVID-19 patients are stratified by anosmia or ageusia presentation support the idea that these symptoms are very characteristic in COVID-19 patients and a key in viral neurotropism. Hence, headache and dizziness might not be due to CNS viral invasion, and instead anosmia and ageusia are. Nevertheless, it must be kept in mind that neurological manifestations

observed in COVID-19 do not always imply CNS viral invasion and these are just possible hypothesis to justify our results.

In the current study, the differences observed could be the sequelae of much larger changes during the acute period of the disease, similarly to what occurs in neurologic symptoms in COVID-19. In this regard, recent reports have noted a high recovery rate of olfactory function 1–2 weeks after the onset of the symptoms.[33] However, on objective tests in a study, 80% of the patients who reported spontaneous regression of anosmia and ageusia still presented a certain degree of residual dysfunction.[12] This suggests that although neural damage may improve substantially after COVID-19 symptoms have ceased, subtle changes may still remain.

Several limitations of our study must be addressed. Firstly, this is a cross-sectional study and there is no ophthalmological evaluation at earlier stages of the disease due to the emergency situation, risk of contagion, poor general health condition in some cases, and exploration of contagious patients in the Ophthalmology clinic was avoided to reduce the risk of cross-infection. Therefore, the ophthalmological examination was performed 4 weeks after COVID-19 diagnosis because the patients had to comply with the 14-day period of mandatory isolation. A within-patient-comparison during and after COVID infection would be of great value, but great limitations apply. In addition, only refractive error was considered to exclude those with refractive error greater than 6 diopters, but axial length was not measured. The patient group was heterogeneous regarding the patients' general history and disease severity, which could explain the difficulties in identifying associations with the clinical variables.

Consequences of SARS-CoV-2 neurologic invasion and the effect of this neurotropic virus on the optic nerve and the retina are still unknown. This is the most complete study to date assessing structural changes in the retina and optic nerve of recovered COVID-19 patients using OCT technology. In the current study we prove optic nerve involvement in SARS-CoV-2 infection, although we cannot prove the exact mechanism of these changes. Hence, the inflammation caused by the virus would account for the thickening of some layers and the atrophy of other layers could be a result of transinaptic damage.

In conclusion, we found features related to optic nerve involvement in recovered COVID-19 patients. SARS-CoV-2 may be a neurotropic virus and affect the optic nerve, the olfatory bulb being the main entry pathway of the virus to the CNS. These alterations in the retinal layers may represent residual inflammation of the acute illness, transient changes, or long-term sequelae and the clinical significance of these findings is unknown. Therefore, larger and long-term follow-up studies including subgroups would make valuable contributions.

Acknowledgements

We would like to thank the patients who participated in this study. We are also grateful to Ana Gonzalez Alvarez-Nava, Helga Tallon Avila, Maria Isabel Sanchez Perea, Maria Carmen Rivera Sequera, and the investigators of COVID-19_URG-HCSC Register: Juan González del Castillo, Adrián Valls Carbó, Enrique del Toro, Eduardo Cardassay, Gabriel Cozar López, María del Mar Suárez-Cadenas, Pablo Jerez Fernández, Beatriz Angós, Cristina Díaz del Arco, Esther Rodríguez Adrada, María Teresa Montalvo Moraleda, Carolina Espejo Paeres, Amanda López Picado, Carmen Martínez Valero, Juan de D. Miranda, David Chaparro, Miguel Ángel García Briñón, José Luis Fernández Rueda, José María Leal Pozuelo, José Luis Fernández Rueda, Víctor Hernández Martín-Romo.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Barbara Burgos-Blasco https://orcid.org/0000-0003-2178-6164

Noemi Güemes-Villahoz https://orcid.org/0000-0002-9289-1212

BeatrizVidal-Villegas https://orcid.org/0000-0001-9352-1400

Juan Donate-Lopez https://orcid.org/0000-0002-9944-6736

References

1. Ren L-L, Wang Y-M, Wu Z-Q, et al. Identification of a novel coronavirus causing severe pneumonia in human. *Chin Med J (Engl)* 2020; 133(9): 1015 1024.
2. Larici AR, Cicchetti G, Marano R, et al. Multimodality imaging of COVID-19 pneumonia: from diagnosis to follow-up. A comprehensive review. *Eur J Radiol* 2020; 131: 109217.
3. Guan W, Ni Z, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020; 382: 1708 1720.
4. Vabret N, Britton GJ, Gruber C, et al. Immunology of COVID-19: current state of the science. *Immunity* 2020; 52(6): 910 941.
5. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol* 2020; 77: 683 690.
6. Ahmad I and Rathore FA. Neurological manifestations and complications of COVID-19: a literature review. *J Clin Neurosci* 2020; 77: 8 12.
7. Güemes-Villahoz N, Burgos-Blasco B, García-Feijoó J, et al. Conjunctivitis in COVID-19 patients: frequency and clinical presentation. *Graefes Arch Clin Exp Ophthalmol* 2020; 258: 2501 2507.

8. Seah I and Agrawal R. Can the coronavirus disease 2019 (COVID-19) affect the eyes? A review of coronaviruses and ocular implications in humans and animals. *Ocul Immunol Inflamm* 2020; 28(3): 391 395.
9. Burgos-Blasco B, Guemes-Villahoz N, Donate-Lopez J, et al. Optic nerve analysis in COVID-19 patients. *J Med Virol.* 2021; 93(1): 190 191.
10. Medeiros FA, Zangwill LM, Bowd C, et al. Evaluation of retinal nerve fiber layer, optic nerve head, and macular thickness measurements for glaucoma detection using optical coherence tomography. *Am J Ophthalmol* 2005; 139(1): 44 55.
11. Lamirel C, Newman NJ and Biousse V. Optical coherence tomography (OCT) in optic neuritis and multiple sclerosis. *Rev Neurol (Paris)* 2010; 166(12): 978 986.
12. Vaira LA, Deiana G, Fois AG, et al. Objective evaluation of anosmia and ageusia in COVID-19 patients: single-center experience on 72 cases. *Head Neck* 2020; 42: 1252 1258.
13. Tong JY, Wong A, Zhu D, et al. The prevalence of olfactory and gustatory dysfunction in COVID-19 patients: a systematic review and meta-analysis. *Otolaryngol Head Neck Surg* 2020; 163(1): 3 11.
14. Gómez-Iglesias P, Porta-Etessam J, Montalvo T, et al. An online observational study of patients with olfactory and gustory alterations secondary to SARS-CoV-2 infection. *Front Public Health* 2020; 8: 243.
15. Invernizzi A, Torre A, Parrulli S, et al. Retinal findings in patients with COVID-19: results from the SERPICO-19 study. *EClinicalMedicine* 2020; 27: 100550.
16. Savastano A, Crincoli E, Savastano M, et al. Peripapillary retinal vascular involvement in early post-COVID-19 patients. *J Clin Med* 2020; 9(9): 2895.
17. McGavern DB and Kang SS. Illuminating viral infections in the nervous system. *Nat Rev Immunol* 2011; 11(5): 318 329.
18. Bernard-Valnet R, Pizzarotti B, Anichini A, et al. Two patients with acute meningo-encephalitis concomitant to SARS-CoV-2 infection. *medRxiv*. DOI: 10.1101/2020.04 .17.20060251.
19. Moriguchi T, Harii N, Goto J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis* 2020; 94: 55 58.
20. Pereira A. Long-term neurological threats of COVID-19: a call to update the thinking about the outcomes of the coronavirus pandemic. *Front Neurol* 2020; 11: 2019 2021.
21. Santos SF, de Oliveira HL, Yamada ES, et al. The gut and Parkinson's Disease: a bidirectional pathway. *Front Neurol* 2019; 10: 574.
22. Aydin TS, Umit D, Nur OM, et al. Optical coherence tomography findings in Parkinson's disease. *Kaohsiung J Med Sci* 2018; 34(3): 166 171.
23. Satue M, Obis J, Alarcia R, et al. Retinal and choroidal changes in patients with Parkinson's disease detected by swept-source optical coherence tomography. *Curr Eye Res* 2018; 43(1): 109 115.
24. Garcia-Martin E, Larrosa JM, Polo V, et al. Distribution of retinal layer atrophy in patients with Parkinson disease and association with disease severity and duration. *Am J Ophthalmol* 2014; 157(2): 470 478.e2.
25. Hasanov S, Demirkilinc Biler E, Acarer A, et al. Functional and morphological assessment of ocular structures and follow-up of patients with early-stage Parkinson's disease. *Int Ophthalmol* 2019; 39(6): 1255 1262.
26. Singh M, Khan RS, Dine K, et al. Intracranial inoculation is more potent than intranasal inoculation for inducing optic neuritis in the mouse hepatitis virus-induced model of multiple sclerosis. *Front Cell Infect Microbiol* 2018; 8: 311.
27. Gu J, Gong E, Zhang B, et al. Multiple organ infection and the pathogenesis of SARS. *J Exp Med* 2005; 202(3): 415 424.
28. Zhou L, Zhang M, Wang J, et al. Sars-Cov-2: underestimated damage to nervous system. *Travel Med Infect Dis* 2020; 36: 101642.
29. Perlman S, Evans G and Afifi A. Effect of olfactory bulb ablation on spread of a neurotropic coronavirus into the mouse brain. *J Exp Med* 1990; 172(4): 1127 1132.
30. Pouga L. Encephalitic syndrome and anosmia in COVID-19: do these clinical presentations really reflect SARS-CoV-2 neurotropism? A theory based on the review of 25 COVID-19 cases. *J Med Virol*. Epub ahead of print 16 July 2020. DOI: 10.1002/jmv.26309.
31. Casagrande M, Fitzek A, Püschel K, et al. Detection of SARS-CoV-2 in human retinal biopsies of deceased COVID-19 patients. *Ocul Immunol Inflamm* 2020; 28(5): 721 725.
32. Li M-Y, Li L, Zhang Y, et al. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infect Dis Poverty* 2020; 9(1): 45.
33. Lechien JR, Chiesa-Estomba CM, De Siati DR, et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Oto-Rhino-Laryngology* 2020; 277: 2251 2261.

5.4 REDUCED MACULAR VESSEL DENSITY IN COVID-19 PATIENTS WITH AND WITHOUT ASSOCIATED THROMBOTIC EVENTS USING OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY

Graefe's Archive for Clinical and Experimental Ophthalmology
https://doi.org/10.1007/s00417-021-05186-0

RETINAL DISORDERS

Reduced macular vessel density in COVID-19 patients with and without associated thrombotic events using optical coherence tomography angiography

Noemi Guemes-Villahoz[1] · Barbara Burgos-Blasco[1] · Beatriz Vidal-Villegas[1] · Juan Donate-López[2] · María Herrera de la Muela[3] · Lorenzo López-Guajardo[2] · Francisco Javier Martín-Sánchez[4] · Julián García-Feijoó[5]

Received: 20 November 2020 / Revised: 15 March 2021 / Accepted: 7 April 2021

Abstract

Purpose Thrombotic events (TE) represent one of the major complications of SARS-CoV-2 infection. The objective is to evaluate vessel density (VD) and perfusion density (PD) by optical coherence tomography angiography (OCTA) in COVID-19 patients, and compare the findings with healthy controls. The secondary objective is to evaluate if there are differences in OCTA parameters between COVID-19 patients with and without associated TE.

Methods Cross-sectional case–control study that included patients with laboratory-confirmed diagnosis of COVID-19 with and without TE related to the infection and age-matched healthy controls. Ophthalmological examination and OCTA were performed 12 weeks after diagnosis. Demographic data and medical history were collected. Macular OCTA parameters in the superficial retinal plexus were analyzed according to ETDRS sectors.

Results Ninety patients were included, 19 (20%) COVID-19 patients with associated TE, 47 (49.5%) COVID-19 patients without TE, and 29 (30.5%) healthy controls. Fifty-three (55.7%) were male, mean age 54.4 (SD 10.2) years. COVID-19 patients presented significantly lower VD than healthy controls: central ($p=0.003$), inner ring ($p=0.026$), outer ring ($p=0.001$). PD was also significantly decreased: outer ring ($p=0.003$), full area ($p=0.001$). No differences in OCTA parameters were found between COVID-19 patients with and without TE.

Conclusions OCTA represents a promising tool for the in vivo assessment of microvascular changes in COVID-19. Patients with SARS-CoV-2 infection show lower VD and PD compared to healthy controls. However, no differences were found between COVID-19 when considering TE. Prospective studies are required to further evaluate the retinal microvascular involvement of SARS-CoV-2 and its impact on the vasculature of other organs.

✉ Noemi Guemes-Villahoz
noemiguemes@gmail.com

[1] Ophthalmology Department, Hospital Clínico San Carlos, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain

[2] Ophthalmology Department, Hospital Clínico San Carlos, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Oftared, Madrid, Spain

[3] Obstetrics and Gynecology Department, Instituto de Salud de La Mujer, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IsISSC), Madrid, Spain

[4] Department of Emergency, Hospital Clinico San Carlos, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain

[5] Ophthalmology Department, Hospital Clínico San Carlos, Departamento de Inmunología, Oftalmología y ORL, Facultad de Medicina, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Oftared, Madrid, Spain

Published online: 07 May 2021

Key messages

- Optical coherence angiography (OCTA) represents a promising tool in the in vivo study of microvascular involvement in COVID-19 patients.
- Patients with SARS-CoV-2 infection show reduced vessel density and perfusion density compared to healthy controls.
- OCTA parameters showed no difference between COVID-19 patients when considering the history of COVID-related thrombotic events.
- Retinal vasculature imaging with OCTA may provide a potential biomarker of vascular changes related to SARS-CoV-2 in other organs.

Keywords COVID-19 · SARS-CoV-2 · OCTA · Retina · Thrombotic events

Introduction

Coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has reached pandemic proportions in an unprecedented time frame. Since its emergence, a number of associated complications have been described that may occur during both the acute and late phases of the disease. Among these complications, the development of thrombotic events (TE), such as pulmonary embolism (PE) and deep vein thrombosis (DVT), has been described [1, 2]. The actual mechanisms of COVID-19-induced thrombosis have not been elucidated, although it is speculated that a causal and bidirectional relationship between inflammation and thrombosis may be the origin of these phenomena [3].

Optical coherence tomography angiography (OCTA) is a novel and non-invasive technique that generates a three-dimensional angiogram of the retina, allowing a quantitative evaluation of retinal blood vessels without the need for intravenous contrast [4], which makes it a promising technique in the study of microangiopathies and thrombotic phenomena related to COVID-19. Compared to qualitative evaluations, quantitative objective measurements of retinal vascularization offer the possibility of early and accurate detection of subtle microvascular abnormalities not clinically detectable.

Since retinal vascularization shares morphological and pathophysiological characteristics with the vasculature of other organs, the study of retinal microvasculature by OCTA has been used to evaluate other systemic diseases, including cardiovascular and infectious diseases [5–7]. Therefore, the assessment of retinal vascularization in COVID-19 patients is of considerable value, especially in those who have suffered vascular complications associated with the infection. To the best of our knowledge, no studies evaluating retinal circulation by OCTA in COVID-19 patients who developed TE related to the disease have been published to date.

The aim of this study is to evaluate the vessel density and perfusion density by OCTA in COVID-19 patients, and to compare the findings with healthy controls. The secondary objective is to evaluate if there are differences in OCTA parameters between COVID-19 patients when considering the history of TE.

Methods

Subjects

Cross-sectional case–control study was conducted at the Hospital Clinico San Carlos (HCSC) in Madrid, Spain. The study was approved by the HCSC's Clinical Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

The study selected 3 groups of patients: patients with confirmed diagnosis of COVID-19 who presented TE associated to the infection (group 1), patients with confirmed diagnosis of COVID-19 without TE (group 2), and healthy controls (group 3). The groups were matched by age, sex, and refraction.

For group 1, patients with laboratory-confirmed diagnosis of COVID-19 who presented TE associated with the disease were selected from a cohort of patients attended at HCSC from March 23 to May 31, 2020. The inclusion criteria for group 1 were as follows: age 18 to 70 years, positive reverse transcriptase polymerase chain reaction (RT-PCR) test from nasopharyngeal swab for SARS-CoV-2, TE presented during the illness in the absence of any disease, or coagulation disorder that might justify it. The TE considered

were pulmonary embolism (PE) in a patient with COVID-19 pneumonia confirmed by computed tomography angiography and/or deep vein thrombosis confirmed by ultrasound. The criteria also included blood tests performed during hospital admission and written informed consent for study participation. Critically ill patients requiring admission to the intensive care unit (ICU), those with concomitant eye diseases, quarantined patients, those unable to attend to the hospital, or lacking consent to participate in the study were excluded.

Group 2 included patients who attended the emergency department (ED) of HCSC from March 23 to 26, 2020, and presented a laboratory-confirmed diagnosis of SARS-CoV-2 infection. The inclusion and exclusion criteria for this group were the same as for group 1, except for the presence of TE related to COVID-19.

The control group (group 3) included a cohort of healthy patients who attended the ophthalmology department for routine eye examinations. Inclusion criteria for this group were as follows: age 18 to 70 years, negative laboratory tests for SARS-CoV-2 infection (IgG and IgM-ELISA serology and PCR from nasopharyngeal swab, both negative), absence of symptoms compatible with COVID-19 or close contact with COVID-19 patient in the 14 days prior to the evaluation, absence of concomitant eye diseases, and written informed consent for participation in the study.

Eye diseases excluded for all three groups were high myopia (> 6 diopters), retinal vascular disease, macular and optic nerve disease, previous ocular surgery other than uncomplicated cataract surgery performed at least 6 months prior to the evaluation, and media opacity affecting OCTA's scan or image quality.

Demographic data, medical history (arterial hypertension, diabetes mellitus, dyslipidemia, obesity, chronic respiratory disease and cardiac disease, smoking), clinical evaluation data (onset of symptoms, oxygen saturation upon arrival, consolidation on chest X-ray, and clinical severity according to the WHO ordinal scale) [8], and laboratory test results (complete hemogram, lipid profile, C-reactive protein (PCR), troponins, ferritin, fibrinogen, lactate dehydrogenase levels (LDH), and D-dimer (DD)) during the hospital stay were collected. As for the laboratory tests, the highest levels documented during the hospital stay were considered.

Ophthalmic examination

Eye exam included a slit lamp examination of the anterior segment, a fundus examination and OCTA 12 weeks after the diagnosis of the infection. All procedures performed followed infection prevention and control measures according to hospital's protocol.

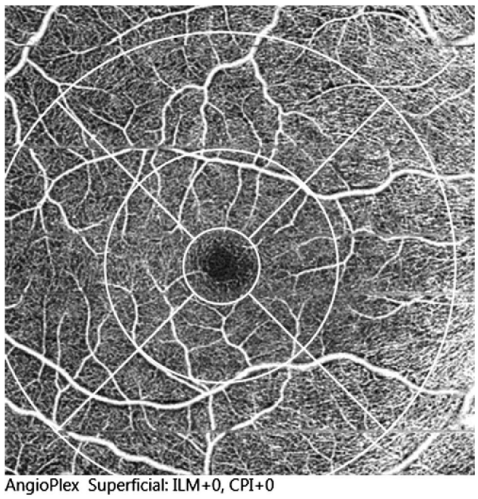

Fig. 1 OCTA of the macular region in the superficial capillary plexus, depicting the 9 sectors of the ETDRS grid. Central circle and inner and outer rings, which are further subdivided into superior, nasal, inferior and temporal regions

OCTA images were obtained using the Zeiss Cirrus 5000 spectral domain OCTA with AngioPlex (Carl Zeiss Meditec, Inc., Dublin, CA, USA). Macular angiography images were performed using the 6×6 mm macular cube protocol in both eyes for each subject. The inclusion criteria for acceptable signal strength (SS) was 7 or more. The complex optical microangiography (cOMAG) algorithm analyzed changes in the complex signals. The results were analyzed using Cirrus OCTA software (AngioPlex™, version 11.0).

Eye exam was performed by two ophthalmologists (NG and BB). OCTA was performed by the same examiner, and reviewed individually by the same two ophthalmologists for quality assessment, excluding those images with lower quality. OCTA data of the superficial capillary plexus (SCP) were collected, including vessel density (VD), perfusion density (PD), and the area, perimeter, and morphology (circularity) of the foveal avascular zone (FAZ). VD was defined as the total length of perfused vasculature per unit area in the region of measurement and PD was defined as the total area of perfused vasculature per unit area in the same region. The built-in analytic algorithm automatically outlined the FAZ boundary along the innermost capillaries, quantifying the area and perimeter of this zone. FAZ circularity was defined as $4\pi A/P$, where A was the area and P was the perimeter.

The macular region was segmented according to the nine sectors of the Study of Early Treatment of Diabetic Retinopathy (ETDRS). The fovea was represented by a

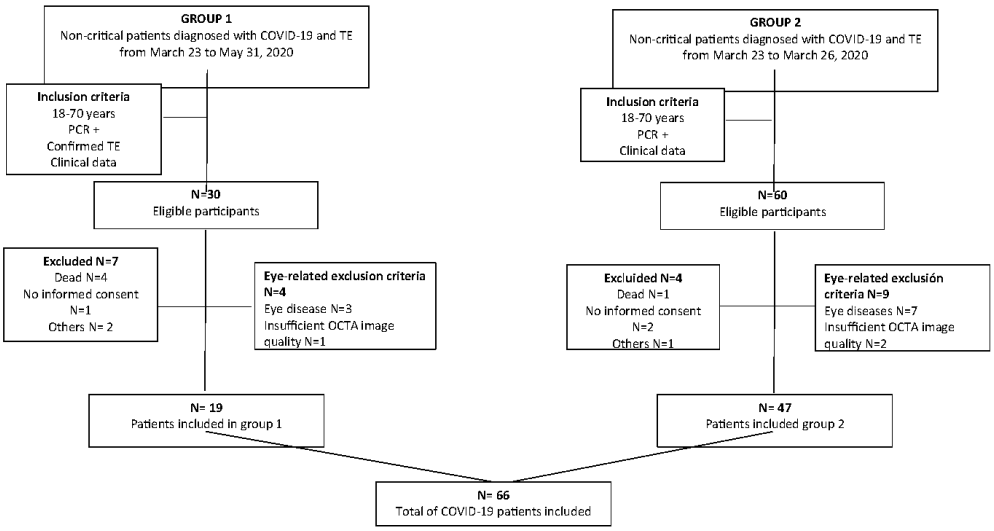

Fig. 2 Flowchart of COVID-19 patients included in the study

central circle of 1 mm in diameter. The inner ring had an inner diameter of 1 mm and an outer diameter of 3 mm, and the outer circle had an inner diameter of 3 mm and an outer diameter of 6 mm (Fig. 1). Both inner and outer rings were further subdivided into upper, nasal, lower, and temporal regions. The subjects' right eye was included, unless it did not meet the criteria for inclusion and exclusion, in which case the left eye was included.

Statistics

All statistical tests were performed using the software package IBM SPSS (version 25.0; IBM Corp., Somers, NY, USA). Demographic and clinical data of the COVID-19 patients is presented as mean and standard deviation for quantitative variables, while prevalence of categorical variables will be presented as percentage. Differences in age and

Table 1 Clinical characteristics of COVID-19 patients

	Group 1 COVID-19 with TE ($n=19$)	Group 2 COVID-19 without TE ($n=47$)	p
Demographic data			
Age (mean [SD])	57.1 (6.0)	57.3 (8.2)	
Male (n[%])	13 (68.4)	27 (57.4)	0.260
Female (n[%])	6 (31.6)	20 (42.6)	0.260
Medical history			
Arterial hypertension (n[%])	4 (25.0)	15 (31.9)	0.603
Diabetes mellitus (n[%])	5 (31.2)	8 (17.0)	0.224
Dyslipidemia (n[%])	4 (25.0)	15 (31.9)	0.271
Cardiac disease (n[%])	1 (6.2)	4 (8.5)	0.773
Renal disease (n[%])	0 (0)	1 (2.1)	0.556
Liver disease (n[%])	2 (12.5)	1 (2.1)	0.092
Cancer (n[%])	2 (12.5)	3 (6.4)	0.434
Chronic respiratory disease (n[%])	1 (6.2)	3 (6.4)	0.985

sex between COVID-19 patients and the control group were compared using the Mann–Whitney *U* test and the chi^2 test.

The OCT data of both groups are represented as means and standard deviation. The normality of the variables was confirmed with the Kolmogorov–Smirnov test. To analyze the differences in the OCT parameters between COVID-19 patients and healthy controls, a *t*-student test was performed. Likewise, to compare the group of patients with and without TE, a *t*-student test was performed. Statistical significance was set at $p < 0.05$.

Results

The total study population included 95 patients, 66 (69.4%) COVID-19 patients, and 29 (30.5%) healthy controls. Among the 66 COVID-19 patients, 19 (28.8%) belonged to group 1 and 47 (71.2%) to group 2. Of the non-critically ill patients diagnosed with COVID-19 and TE from March 23 to May 31, 2020, 30 patients met the inclusion criteria. Among these, 19 patients were finally included in group 1. All of them (19/19) presented PE, and 2 (2/19) presented both PE and DVT related to COVID-19. Likewise, of the non-critical COVID-19 patients that attended ED on the dates indicated, 47 patients met the inclusion and exclusion criteria (group 2). Figure 2 shows the flow chart of the cases included in the study.

Demographic and clinical characteristics of COVID-19 patients are summarized in Table 1. The mean age of healthy controls was 51.0 (SD 11.9). There were no statistically significant differences between groups in terms of age and sex.

Eye exam was performed 88 days (86–90) after COVID-19 diagnosis and revealed the ocular diseases previously defined as exclusion criteria in a total of 10 patients, who were excluded from the study. Fundus examination of the 66 patients with a history of SARS-CoV-2 infection was unremarkable. No retinal vascular changes, macular or optic nerve involvement, were evident in any of the groups. Neither did any patient report decreased vision or other noticeable ocular symptoms during the disease, nor until the time of the evaluation.

Significant differences in VD and PD were detected by OCTA between COVID-19 patients and healthy controls. COVID-19 patients presented significantly lower VD than healthy controls: central ($p = 0.003$), inner ring ($p = 0.026$), outer ring ($p = 0.001$). PD was also decreased in COVID-19 patients: outer ring ($p = 0.003$), full area ($p = 0.001$). Table 2 shows macular OCTA data of COVID-19 patients and healthy controls. (Additional information is provided in a supplementary table). However, within the COVID-19 group, no differences in OCTA parameters were found when considering history of TE.

Laboratory parameters evidenced significant differences in D-dimer levels between the group with and without TE ($p = 0.015$). Group 1 revealed mean D-dimer values of 5528.5 (SD 6204.3) and group 2 of 648.2 (SD 401.5).

Table 2 OCTA data in COVID-19 patients and healthy controls

Variable	COVID-19 ($N = 66$)		Healthy controls ($N = 29$)		*p*
	Mean	SD	Mean	SD	
VD central	7.77	3.26	10.10	3.41	**0.003**
VD inner	16.49	2.47	27.52	1.82	**0.026**
VD outer	17.28	2.15	18.48	1.21	**0.001**
VD complete	16.89	2.02	17.72	2.49	0.118
PD central	20.05	25.35	22.41	7.84	0.494
PD inner	39.18	6.76	56.17	75.30	0.235
PD outer	42.94	5.65	45.79	3.22	**0.003**
PD complete	41.46	4.99	44.35	3.30	**0.001**
FAZ a	0.50	1.87	0.12	0.44	0.152
FAZ p	2.10	0.62	2.28	0.68	0.262
FAZ c	0.68	0.11	1.96	3.81	0.106

The significance of the *p* values is in bold ($p < 0.05$)

SD, standard deviation; *VD*, vessel density; *PD*, perfusion density; *FAZ*, foveal avascular zone; *a*, area; *p*, perimeter; *c*, circularity

Discussion

Thrombotic events are emerging as one of the most relevant sequelae of SARS-CoV-2 infection [9, 10]. A high incidence of TE has been described in COVID-19 patients, more so in critically ill patients, reporting variable incidences ranging from 11.5 to 27% [1, 10–14]. Although PE represents the most frequent thrombotic manifestation associated with the infection [1], TE, venous and arterials, have been also described in other organs such as the eye [15]. Our study reveals quantitative differences in retinal microcirculation between COVID-19 patients and healthy controls. Patients with history of SARS-CoV-2 infection had significantly lower vessel density and perfusion density than healthy controls 3 months after the acute phase of the infection. These findings provide relevant information about the microvascular involvement of SARS-CoV-2 infection and the possible vascular sequelae of COVID-19. Nevertheless, no differences in these parameters have been detected in COVID-19 patients when considering the history of TE, which may indicate that the microvascular involvement of SARS-CoV-2 at the retinal level is not conditioned by the presence of TE at other levels. Similarly, no increased incidence of retinal vascular events has been found in COVID-19 patients who had TE, compared to those who did not.

OCTA is a novel technique that allows the study and objective quantification of retinal vasculature, assessing the retinal microvascular impact of the disease. The fact that the

VD and PD are significantly reduced in COVID-19 patients even 3 months after the acute phase of the infection exhibits important questions about the mechanism of microvascular damage of SARS-CoV-2 and its possible mid- and long-term sequels, both in the eye and other organs. To date, the mechanism by which SARS-CoV-2 may affect the retina vasculature is unknown. Recently, SARS-CoV-2 RNA has been detected in retinal biopsies performed postmortem in patients with COVID-19 [16]. The detection of the virus in intraocular tissue could be explained by the presence of angiotensin-converting enzyme 2 (ACE2), the main receptor of SARS-CoV-2 cellular entry, in the retina [17]. In this sense, the presence of ACE2 has also been detected in arterial and venous endothelial cells [18]. It then seems feasible that vascular changes may occur at this level.

Furthermore, thrombosis and extrapulmonary manifestations have been reported without confirmed virus presence at these sites [19], suggesting that SARS-CoV-2 may cause both direct cytopathic damage and indirect damage related to the intense inflammatory response and hypercoagulable state it induces. These findings have yielded to the concept of thrombo-inflammation as key phenomena in the pathophysiology of COVID-19, whereby inflammation activates coagulation, and coagulation heightens inflammatory activity [20, 21]. This rationale is consistent with the findings reported in autopsies of COVID-19 patients who have died from the disease, which have revealed microvasculature thrombi as prominent feature in multiple organs [22]. All these COVID-19-related vascular phenomena might partly explain the findings encountered in our study.

The post-mortem examination for further evaluation of the vascular damage in the lungs and other organs is supported. However, retinal circulation has the unique advantage of being easily studied in vivo. Hence, OCTA may provide a valuable tool in the study of microvascular involvement in COVID-19 patients, since it may represent a potential biomarker of vascular damage in other organs [5, 23, 24].

Current evidence about the presence of retinal venous thrombosis in COVID-19 patients is scarce. In this sense, it has been reported a case of a 52-year-old male without cardiovascular risk factors, and normal coagulation study who presented a branch retinal vein occlusion 10 days after the onset of fever and malaise with subsequent laboratory confirmation of SARS-CoV-2 infection [15]. Another case of a 54-year-old woman with COVID-19-associated pneumonia and impending retinal vein occlusion has also been recently described. She presented decreased visual acuity and retinal changes compatible with impending central retinal vein occlusion, which resolved after treatment and resolution of the pneumonia [25]. All this suggests that the hypercoagulability state associated with COVID-19 may also clinically affect retinal vasculature.

Our study has several limitations. First, this study did not include critically ill patients requiring ICU admission. Since the incidence of TE in critical COVID-19 patients is much higher, clinical and subclinical findings might appear more evident in this group of patients. In addition, critically ill patients present progressive and sustained hypoxia that has been associated with an exaggerated release of proinflammatory and prothrombotic factors [26, 27], which could aggravate the changes found in retinal microcirculation. On the other hand, the eye exam was performed 12 weeks after COVID-19 diagnosis, so certain retinal vascular changes that might occur during the acute phase of the infection could not be clinically present in the subsequent examination. Although it would have been interesting to evaluate patients during the acute phase of infection, the extremely critical scenario and hospital logistics prevented such evaluation during the peak of the pandemic. Finally, the number of patients recruited in the study is limited. Prospective studies with larger numbers of patients may yield more data about the microvascular impact of SARS-CoV-2 infection.

In conclusion, the evaluation of retinal circulation represents a promising tool in the in vivo study of microvascular involvement in COVID-19 patients. Patients with SARS-CoV-2 infection show decreased retinal vessel density and perfusion density compared to healthy controls as late as 12 weeks after the acute phase of infection. However, no differences in OCTA parameters have been found between COVID-19 patients when considering the history of TE. These findings provide relevant information about the microvascular damage of SARS-CoV-2, even in the absence of TE, and its possible impact on the vasculature of other organs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00417-021-05186-0.

Acknowledgements We wish to thank participating investigators that helped collecting data: Ana González Álvarez-Nava, Helga Tallon Ávila, María Isabel Sánchez Perea, María Carmen Rivera Sequera. COVID-19_URG-HCSC Register: Juan González del Castillo, Adrián Valls Carbó, Enrique del Toro, Eduardo Cardassay, Gabriel Cozar López, María del Mar Suárez-Cadenas, Pablo Jerez Fernández, Beatriz Angós, Cristina Díaz del Arco, Esther Rodríguez Adrada, María Teresa Montalvo Moraleda, Carolina Espejo Paeres, Amanda López Picado, Carmen Martínez Valero, Juande D. Miranda, David Chaparro, Miguel Ángel García Briñón, José Luis Fernández Rueda, José Mª Leal Pozuelo, José Luis Fernández Rueda, Víctor Hernández Martín-Romo.

Author contribution All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article and take responsibility for the integrity of the work as a whole.

Data Availability Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval The study was approved by the Clinical Research Ethics Committee of HCSC and was conducted in accordance with the Helsinki Declaration.

Consent to participate Written informed consent was obtained from all patients.

Consent for publication All authors have given their approval for this version to be published.

Conflict of interest The authors declare no competing interests.

References

1. Llitjos J-F, Leclerc M, Chochois C et al (2020) High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thromb Haemost 18:1743–1746. https://doi.org/10.1111/jth.14869
2. Demelo-Rodríguez P, Cervilla-Muñoz E, Ordieres-Ortega L et al (2020) Incidence of asymptomatic deep vein thrombosis in patients with COVID-19 pneumonia and elevated D-dimer levels. Thromb Res 192:23–26. https://doi.org/10.1016/j.thromres.2020.05.018
3. Abou-Ismail MY, Diamond A, Kapoor S et al (2020) The hypercoagulable state in COVID-19: incidence, pathophysiology, and management. Thromb Res 194:101–115. https://doi.org/10.1016/j.thromres.2020.06.029
4. Hagag A, Gao S, Jia Y, Huang D (2017) Optical coherence tomography angiography: technical principles and clinical applications in ophthalmology. Taiwan J Ophthalmol 7:115. https://doi.org/10.4103/tjo.tjo_31_17
5. Pascual-Prieto J, Burgos-Blasco B, Ávila Sánchez-Torija M et al (2020) Utility of optical coherence tomography angiography in detecting vascular retinal damage caused by arterial hypertension. Eur J Ophthalmol 30:579–585. https://doi.org/10.1177/1120672119831159
6. Akmaz B, Akay F, Güven YZ et al (2020) The long-term effect of human immunodeficiency virus infection on retinal microvasculature and the ganglion cell-inner plexiform layer: an OCT angiography study. Graefes Arch Clin Exp Ophthalmol 258:1671–1676. https://doi.org/10.1007/s00417-020-04749-x
7. Patton N, Aslam T, Macgillivray T et al (2005) Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J Anat 206:319–348. https://doi.org/10.1111/j.1469-7580.2005.00395.x
8. WHO. Ordinal Scale COVID-19. https://www.who.int/blueprint/priority-diseases/key-action/COVID-19_Treatment_Trial_Design_Master_Protocol_synopsis_Final_18022020.pdf. Accessed 24 Oct 2020
9. Miró Ò, Llorens P, Aguirre A et al (2020) Association between Covid-19 and pulmonary embolism (AC-19-PE study). Thromb Res 196:322–324. https://doi.org/10.1016/j.thromres.2020.09.010
10. Benito N, Filella D, Mateo J et al (2020) Pulmonary Thrombosis or embolism in a large cohort of hospitalized patients with Covid-19. Front Med 7:557. https://doi.org/10.3389/fmed.2020.00557
11. Rali P, O'Corragain O, Oresanya L, et al (2020) Incidence of venous thromboembolism (VTE) in COVID-19: an experience from a single large academic center. J Vasc Surg Venous Lymphat Disord. https://doi.org/10.1016/j.jvsv.2020.09.006
12. Al-Ani F, Chehade S, Lazo-Langner A (2020) Thrombosis risk associated with COVID-19 infection A scoping review. Thromb Res 192:152–160. https://doi.org/10.1016/j.thromres.2020.05.039
13. Griffin DO, Jensen A, Khan M et al (2020) Pulmonary embolism and increased levels of d-dimer in patients with coronavirus disease. Emerg Infect Dis 26:1941–1943. https://doi.org/10.3201/eid2608.201477
14. Giorgi-Pierfranceschi M, Paoletti O, Pan A, et al (2020) Prevalence of asymptomatic deep vein thrombosis in patients hospitalized with SARS-CoV-2 pneumonia: a cross-sectional study. Intern Emerg Med. https://doi.org/10.1007/s11739-020-02472-3
15. Sheth JU, Narayanan R, Goyal J, Goyal V (2020) Retinal vein occlusion in COVID-19: a novel entity. Indian J Ophthalmol 68:2291–2293. https://doi.org/10.4103/ijo.IJO_2380_20
16. Casagrande M, Fitzek A, Püschel K et al (2020) Detection of SARS-CoV-2 in human retinal biopsies of deceased COVID-19 Patients. Ocul Immunol Inflamm 28:721–725. https://doi.org/10.1080/09273948.2020.1770301
17. deS Senanayake P, Drazba J, Shadrach K, et al (2007) Angiotensin II and its receptor subtypes in the human retina. Invest Ophthalmol Vis Sci 48:3301–11. https://doi.org/10.1167/iovs.06-1024
18. Hamming I, Timens W, Bulthuis MLC et al (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 203:631–637. https://doi.org/10.1002/path.1570
19. Sekhawat V, Green A, Mahadeva U (2021) COVID-19 autopsies: conclusions from international studies. Diagn Histopathol (Oxf) 27:103–107. https://doi.org/10.1016/j.mpdhp.2020.11.008
20. Levi M, van der Poll T, Büller HR (2004) Bidirectional relation between inflammation and coagulation. Circulation 109:2698–2704. https://doi.org/10.1161/01.CIR.0000131660.51520.9A
21. Gris J-C, Perez-Martin A, Quéré I, Sotto A (2020) COVID-19 associated coagulopathy: the crowning glory of thrombo-inflammation concept. Anaesthesia Crit Care Pain Med 39:381–382. https://doi.org/10.1016/j.accpm.2020.04.013
22. Carsana L, Sonzogni A, Nasr A et al (2020) Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. Lancet Infect Dis 20:1135–1140. https://doi.org/10.1016/S1473-3099(20)30434-5
23. Lee C-W, Cheng H-C, Chang F-C, Wang A-G (2019) Optical coherence tomography angiography evaluation of retinal microvasculature before and after carotid angioplasty and stenting. Sci Rep 9:14755. https://doi.org/10.1038/s41598-019-51382-8
24. Lee J-Y, Kim JP, Jang H et al (2020) Optical coherence tomography angiography as a potential screening tool for cerebral small vessel diseases. Alzheimers Res Ther 12:73. https://doi.org/10.1186/s13195-020-00638-x
25. Invernizzi A, Pellegrini M, Messenio D, et al (2020) Impending central retinal vein occlusion in a patient with coronavirus disease 2019 (COVID-19). Ocul Immunol Inflamm 1–3. https://doi.org/10.1080/09273948.2020.1807023
26. Somers VK, Kara T, Xie J (2020) Progressive hypoxia. Mayo Clin Proc 95:2339–2342. https://doi.org/10.1016/j.mayocp.2020.09.015
27. Kashani KB (2020) Hypoxia in COVID-19: Sign of severity or cause for poor outcomes. Mayo Clin Proc 95:1094–1096. https://doi.org/10.1016/j.mayocp.2020.04.021

5.5 REDUCED RETINAL VESSEL DENSITY IN COVID-19 PATIENTS AND ELEVATED D-DIMER LEVELS DURING THE ACUTE PHASE OF THE INFECTION

Med Clin (Barc). (2021);156(11):541–546

MEDICINA CLINICA

www.elsevier.es/medicinaclinica

Original article

Reduced retinal vessel density in COVID-19 patients and elevated D-dimer levels during the acute phase of the infection

Noemi Guemes-Villahoz[a,*], Barbara Burgos-Blasco[a], Beatriz Vidal-Villegas[a], Juan Donate-López[b], Francisco Javier Martín-Sánchez[c], Jesús Porta-Etessam[d], Lorenzo López-Guajardo[b], José Luis R. Martín[e], Juan Jorge González-Armengol[c], Julián García-Feijoó[f]

[a] Servicio de Oftalmología, Hospital Clínico San Carlos; Instituto de investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain
[b] Servicio de Oftalmología, Hospital Clínico San Carlos; Instituto de investigación sanitaria del Hospital Clínico San Carlos (IdISSC); Universidad Complutense de Madrid (UCM), Madrid, Spain
[c] Servicio de Urgencias, Hospital Clínico San Carlos, Instituto de investigación sanitaria del Hospital Clínico San Carlos (IdiSCC), Universidad Complutense de Madrid, Spain
[d] Servicio de Neurología, Hospital Clínico San Carlos, Instituto de investigación sanitaria del Hospital Clínico San Carlos (IdiSCC), Universidad Complutense de Madrid, Spain
[e] Medical Research Consultant, Simplifying Research Institute, Spain
[f] Profesor y jefe de servicio de Oftalmología, Hospital Clínico San Carlos; Departamento de Inmunología, Oftalmología y ORL, Facultad de Medicina, Universidad Complutense de Madrid (UCM); IdISSC, Madrid, Spain

ARTICLE INFO

Article history:
Received 30 August 2020
Accepted 10 December 2020

Keywords:
SARS-CoV-2
COVID-19
Macular vessel density
Perfusion
Retina
Optical coherence angiography
D-dimer

ABSTRACT

Purpose: To describe macular vessel density and perfusion in COVID-19 patients using coherence tomography angiography (OCTA) and to investigate whether there is a correlation between retinal vascular abnormalities and clinical and laboratory parameters.
Methods: Cross-sectional analysis conducted at the Hospital Clinico San Carlos in Madrid, Spain. Patients with laboratory-confirmed COVID-19 that were attended in the Emergency Department (ED) from March 23 to March 29, 2020 were included. Fundus examination and OCTA were performed 4 weeks after being attended in ED. Macular OCTA parameters were analyzed and correlated with clinical (severity and hypoxemia- oxygen saturation < 92%) and laboratory parameters during hospital stay (D-Dimer-DD, lactate dehydrogenase-LDH and C-reactive protein-CRP).
Results: 80 patients were included, mean age 55(SD9) years old; 46.3% male. We reported macular vessel density and perfusion measurements in COVID-19 patients. Those patients with D-Dimer ≥ 500 ng/ml during SARS-CoV-2 infection had a decrease of central vessel density (mean difference 2.2; 95%CI 0.4–3.9) and perfusion density (mean difference 4.9; 95%CI 0.9–8.9) after the acute phase of COVID-19. These variations of vessel density and perfusion density were not documented in patients with LDH ≥ 500 U/L, CRP ≥ 10 mg/L and hypoxemia.
Conclusions: COVID-19 patients showed short-term retinal vasculature abnormalities which may be related to a prothrombotic state associated with SARS-CoV-2 infection. Since the retinal microvasculature shares many morphological and physiological properties with the vasculature of other vital organs, further research is needed to establish whether patients with increased D-Dimer levels require more careful assessment and follow-up after COVID-19.

* Corresponding author.
E-mail address: noemiguemes@gmail.com (N. Guemes-Villahoz).

Palabras clave:
SARS-CoV-2
COVID-19
Densidad vascular retiniana
Perfusión
Retina
Angiografía de tomografía de coherencia
Dímero D

Disminución de la densidad vascular retiniana en pacientes con COVID-19 y niveles elevados de dímero D durante la fase aguda de la infección

R E S U M E N

Objetivo: Evaluar la densidad vascular (DV) y la perfusión vascular (PV) retiniana en pacientes con COVID-19 mediante una angiografía por tomografía de coherencia óptica (OCTA), e investigar si existe una correlación entre las anomalías vasculares de la retina y los parámetros clínicos y de laboratorio.
Métodos: Análisis transversal realizado en el Hospital Clínico San Carlos, Madrid. Se incluyeron pacientes con diagnóstico confirmado de COVID-19 atendidos en el Servicio de Urgencias (SU) del 23 al 29 de marzo del 2020. Se realizó una exploración oftalmológica y OCTA cuatro semanas después de acudir al SU. Se analizaron los parámetros maculares de OCTA y se correlacionaron con parámetros clínicos (gravedad e hipoxemia-saturación de oxígeno < 92%) y de laboratorio durante la estancia hospitalaria (dímero D [DD], lactato deshidrogenasa [LDH] y proteína C reactiva [CRP].
Resultados: Se incluyeron 80 pacientes, edad media 55 (DE nueve) años; 46,3% hombres. Las personas con DD > 500 ng/mL durante la infección por SARS-CoV-2 tuvieron una disminución de la DV central (diferencia de medias 2,2; IC 95% 0,4 a 3,9) y PV central (diferencia de medias 4,9; IC 95% 0,9 a 8,9) después de la fase aguda de COVID-19. Estas variaciones no se documentaron en los pacientes con LDH > = 500 U/L, CRP > = 10 mg/L y con hipoxemia.
Conclusiones: Los pacientes con COVID-19 mostraron anomalías de la vasculatura retiniana a corto plazo que pueden estar relacionadas con un estado protrombótico asociado con la infección por SARS-CoV-2. Dado que la microvasculatura de la retina comparte muchas propiedades morfológicas y fisiológicas con la vasculatura de otros órganos vitales, es necesario seguir investigando para determinar si los pacientes con niveles elevados de DD requieren una evaluación y un seguimiento más cuidadoso.

Introduction

An outbreak of a novel coronavirus named severe acute respiratory syndrome coronavirus – 2 (SARS-CoV-2) was reported in Wuhan, Hubei Province (China) in December 2019. This virus causes the coronavirus disease 2019 (COVID-19), that is making an extraordinary impact worldwide.[1]

COVID-19 has shown several clinical manifestations at respiratory, gastrointestinal and neurological levels, among others. SARS-CoV-2 infection has also been related to inflammatory and coagulation abnormalities.[2,3] The pathophysiology of these complications is not fully understood, as well, as is the extent of these after the acute phase of the infection.

To the best of our knowledge, there are no previous reports in the medical literature that depict and quantify the short-term small vessel alterations in COVID-19 patients. Given the current situation of SARS-CoV-2 pandemic, identifying retinal vascular changes that are associated with clinical and laboratory parameters that carry an increased risk of systemic complications secondary to infection has relevant implications in the follow-up of these patients and identification of possible short- and long-term sequels of COVID-19.

To this end, the retina is a relatively accessible organ to evaluate and quantify these microvascular changes via direct examination of blood vessels. Optical coherence tomography angiography (OCTA) is a novel, rapid, and non-invasive technique that generates a three-dimensional angiogram of the retina, without the need for a contrast agent.[4] Several studies have evaluated by OCTA the clinical and subclinical retinal microvascular changes associated with systemic diseases, such as diabetes mellitus, systemic arterial hypertension and infectious diseases.[5–8]

The main purpose of this study is to describe retinal vessel density and perfusion in patients with COVID-19 using OCTA and to investigate whether there is a correlation between retinal vascular abnormalities and clinical and laboratory parameters.

Methods

Study design

This is a case series study with cross-sectional analysis that was carried out in Hospital Clinico San Carlos (HCSC), a tertiary, multispecialty metropolitan *teaching hospital* located in Madrid, Spain. The study was approved by the Clinical Research Ethics Committee and was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all patients.

Patient selection

We selected COVID-19 patients attended in Emergency Department (ED) from March 23 to March 29, 2020. The inclusion criteria were: age 18–65 years old, positive reverse transcriptase–polymerase chain reaction (RT-PCR) test from nasopharyngeal swab for SARS-CoV-2, sample blood for a laboratory test required during the clinical evaluation in acute phase of infection and written informed consent for the participation in the study. Those patients with previous history of stroke or thromboembolic events, concomitant ophthalmic diseases, antithrombotic therapy, the requirement for longer quarantine (patients still admitted to COVID-19 unit or intensive care unit, persistence of respiratory symptoms, persistently positive PCR test, close contact of laboratory-confirmed or probable COVID-19 patients after discharge), unable to attend the hospital, or did not consent to participate in the study were excluded. The concomitant ophthalmic diseases excluded were high myopia (>6 diopters), macular disorders, optic nerve head disease, retinal vascular disorders, previous ocular surgery other than uncomplicated cataract extraction and intraocular lens implantation performed more than 6 months before enrollment and media opacity that affected examination.

Ophthalmologic examination

Every patient underwent an ophthalmologic examination that included slit-lamp exam, fundus examination and optical coherence tomography angiography (OCTA) at least 4 weeks after being attended in the ED in order to fully comply mandatory isolation. The isolation criteria according to the World Health Organization (WHO) is 10 days after symptom onset, plus at least 3 additional days without symptoms.[9] All patients underwent the examination at least 28 days after COVID-19 diagnosis, thus ensuring sufficient time. Moreover, those patients who required a longer quarantine period according to different criteria established by the public health department were excluded. All procedures followed infection control and prevention measures according to the hospital's protocol.

OCTA images were obtained using spectral-domain OCTA Zeiss Cirrus 5000 with AngioPlex (Carl Zeiss Meditec, Inc., Dublin, CA, USA). Macular angiography imaging with 6×6 mm scans was performed in both eyes for each subject. Inclusion criteria for the acceptable signal strength (SS) was 6 or higher. The optical microangiography-complex (OMAG^c) algorithm analyzed the changes in complex signals. Scans were analyzed using the Cirrus OCTA software (AngioPlex^{TM}, version 11.0).

All OCTA scans were performed by the same trained examiner, and were reviewed individually by two ophthalmologists (NG and BB) for quality evaluation, excluding substandard scans. Quantification of the vessel density (VD), perfusion density (PD), and foveal avascular zone (FAZ) area, perimeter, and morphology in the superficial capillary plexus (SCP) were noted. The macular region was segmented according to the nine Early Treatment of Diabetic Retinopathy Study (ETDRS) sectors. The fovea was represented by the central circle of 1 mm in diameter. The inner ring had an inner diameter of 1 mm and an outer diameter of 3 mm, and the outer ring had an inner diameter of 3 mm and outer diameter of 6 mm. Both inner and outer rings were further subdivided into superior, nasal, inferior and temporal region (Figs. 1A and 2B). The right eye was included, unless it did not meet the inclusion and exclusion criteria, in which case the left eye was included.

Variables

Socio-demographic data (age, gender and birthplace), medical history (arterial hypertension, diabetes mellitus, dyslipidemia, obesity, chronic respiratory and heart disease, smoking), clinical findings at ED presentation (symptoms onset, baseline oxygen saturation, consolidation on chest X-ray, and clinical severity according to WHO ordinal scale[10]) and laboratory tests (lymphocytes count, D-Dimer – DD-, C-reactive protein – CRP- and lactate dehydrogenase – LDH-levels) during the hospital stay were collected. Regarding laboratory test, we considered the highest levels documented during the hospital stay.

Statistical analysis

Continuous variables are presented as mean (standard deviation [SD]) or median (interquartile range [IQR]), as appropriate; categorical variables as numbers and percentages. A normality test was carried out for all variables based on their randomness, variance and Kolmogorov–Smirnov test. In the case of not having the possibility of parametric analysis, these were carried out with non-parametric tests to relate the variables under study. Macular OCTA parameters were analyzed according to hypoxemia at ED presentation and the highest values of DD, LDH and CRP. Laboratory data were dichotomized according to cut-off values established in reference COVID-19 studies. Thus, the cutoffs values were 500 ng/ml for DD levels, 10 mg/L for CRP levels and 500 U/L for LDH.[11] Hypoxemia was defined as oxygen saturation < 92%.[12] Comparisons among groups were made using *t*-Student for continuous variables. P value for linear trend was estimated. Subgroup analysis for clinical and laboratory variables was performed. Statistically significant differences were considered two-side *p*-value less than 0.05. The sample size was not calculated as this was an exploratory study. Data analysis was performed using SPSS software, version 24.00 (IBM, New Castle, NY, USA) and STATA version 15.1 (Stata Corp., College Station, TX, USA).

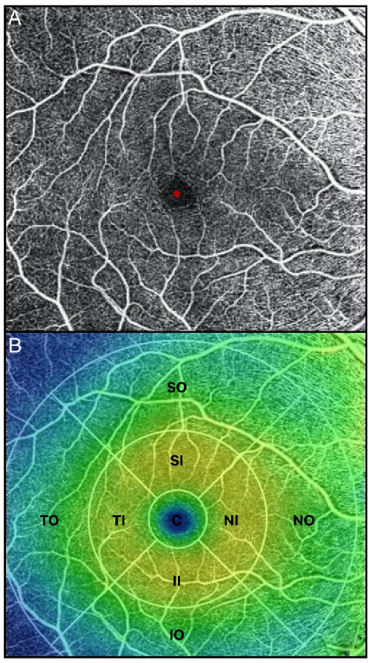

Fig. 1. (A) OCTA of the macular region, showing an angiogram of the retinal circulation in the superficial capillary plexus. The image depicts the retinal arteries and veins within the macular region in a binarized OCTA image, which represents the blood vessels in white color. The central area devoid of vessels represents the foveal avascular zone (*). (B) Color image with ETDRS sectors, depicting the 9 sectors of the ETDRS grid in the macular region. The fovea is represented by the central circle (C). Both inner (I) and outer (O) rings are further subdivided into superior (S), nasal (N), inferior (I) and temporal (T) region.

Results

The overall study population included 80 patients. Of the 584 patients diagnosed with COVID-19 in the Emergency Department from March 23rd to 29th 2020, 235 patients met the inclusion criteria. Of those, 8 patients died, 49 patients were lost to follow-up after emergency department discharge, 22 patients were still admitted to the COVID-19 unit, 15 patients were unable to return to the hospital due to their clinical situation, 12 patients still presented respiratory symptoms after discharge and 11 patients did not give consent. 29 patients had concomitant ocular disorders, which included: high myopia (5), age related macular degeneration (3), glaucoma (3), previous history of central serous choroidopathy (1), history of bilateral pars plana vitrectomy (1), history of retinal vascular disorders (4), moderate-severe media opacities (12). 9 patients were excluded because the OCTA image did not meet

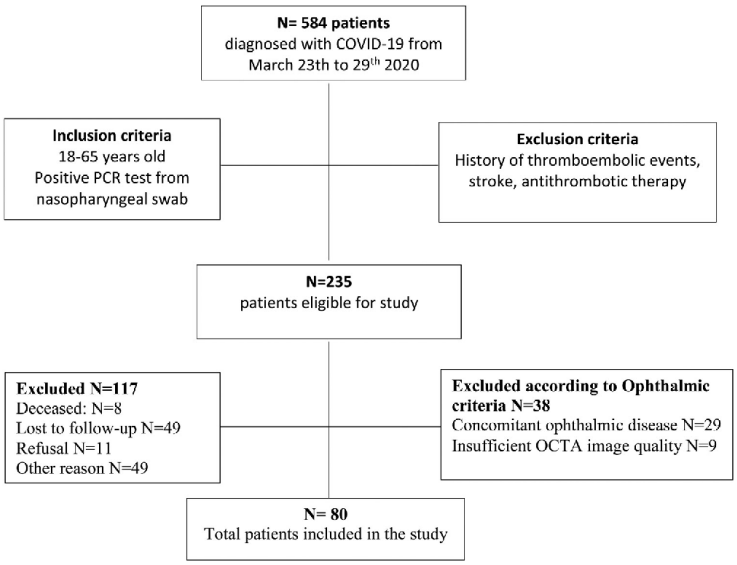

Fig. 2. Flowchart of patient selection.

the quality criteria (signal strength, loss of fixation, segmentation error, and motion artifacts). Fig. 2 depicts the flowchart of patient selection.

Of the 80 patients included, 37 (46.3%) were male, mean age was 54.8 ± 9.2 years. Table 1 depicts the demographical and clinical characteristics of the patients and the laboratory results. Patients were evaluated in the emergency department 7 days (6–10) after the onset of COVID-19 symptoms. Ophthalmic examination was performed 30 days (28–32) after COVID-19 diagnosis. Slit-lamp examination of the anterior segment and the fundus revealed the above-described ophthalmic disorders in 29 patients, who were excluded from the study. Funduscopic examination of the 80 patients included was otherwise unremarkable. None of the patients reported decrease in vision or other remarkable ocular symptoms during the illness, nor until the time of the evaluation.

Vessel density and perfusion density measurements at the SCP of the macular region were analyzed. Table 2 depicts macular OCTA results in COVID-19 patients after the acute phase of the infection. A marked difference was observed for the variable D-dimer. Patients with levels of DD ≥ 500 ng/ml during SARS-CoV-2 infection had a decreased of central vessel density (8.8 (SD 4.0) vs 6.6 (SD 3.6), $p = 0.013$; mean difference 2.2; 95%CI 0.4–3.9) and perfusion 19.6 (SD 9.3) vs. 14.7 (SD 8.0), $p = 0.018$; mean difference 4.9; 95%CI 0.9–8.9) after the acute phase of COVID-19. They also showed a significant decrease of perfusion in the temporal inner sector of the macula (40.3 (SD 5.5) vs. 36.7 (SD 9.5), $p = 0.033$; mean difference 3.6; 95%CI 0.3–6.9) and a tendency in the vessel density in that sector (17.0 (SD 2.0) vs. 15.7 (SD 3.8), $p = 0.053$; mean difference 1.3; 95%CI 0.0–2.6). Fig. 3 represents OCTA parameters according to DD levels. Other OCTA parameters, including FAZ, showed no differences. Blood oxygen saturation levels did not reveal significant differences. The subgroups analysis according to the rest of laboratory parameters (CRP and LDH) did not show a significant difference in OCTA values. Furthermore, no differences were found in patients regarding clinical severity, presence of consolidation on chest x-ray nor comorbidities.

Table 1
Clinical characteristics of COVID-19 patients included in the study.

	N = 80
Demographic data	
Age, years (mean [SD])	54.8 (9.2)
Gender, male (*n*[%])	37 (46.3)
Birthplace, Spain (*n*[%])	46 (57.5)
Medical history	
Current smoker (*n*[%])	4 (5.0)
Arterial hypertension (*n*[%])	20 (25.0)
Diabetes mellitus (*n*[%])	6 (7.5)
Dyslipidemia (*n*[%])	21 (26.3)
Obesity (*n*[%])	8 (10.0)
Chronic respiratory disease (*n*[%])	10 (12.5)
Heart disease (*n*[%])	2 (2.5)
Clinical data	
Time from symptoms onset, days (median [IQR])	7.0 (6.0–10.0)
Oxygen saturation, % (median [IQR])	96 (4)
Oxygen saturation < 92% (n[%])	6 (8)
Chest X-ray, consolidation (n[%])	52 (65.0)
Severity by WHO (n[%])	
Score 1–2	41 (51.3)
Score 3	15 (18.8)
Score ≥ 4	24 (30.0)
Laboraroty data	
Lymphocytes × 10^3/μL (median [IQR])	1.1 (0.8–1.4)
Lymphocytes < 0.1 × 10^3/μL (*n*[%])	29 (36.3)
C-reactive protein, mg/dl (median [IQR])	6.5 (2.1, 12.0)
C-reactive protein > 10 mg/dl (*n*[%])	25 (31.3)
D-Dimer, ng/ml (median [IQR])	613.0 (364.0, 1036.0)
D-Dimer > 500 ng/ml (*n*[%])	48 (60.0)
LDH, U/L (median [IQR])	615.0 (472.0, 823.0)
LDH > 500 U/L (*n*[%])	51 (63.8)

*SD: standard deviation; IQR: interquartil range; WHO: World Health Organization; LDH: lactate dehydrogenase.

Discussion

Although the main clinical manifestations of COVID-19 involve respiratory and gastrointestinal symptoms, there is also evidence of

Table 2
Macular optical coherence tomography angiography results in COVID-19 PATIENTS.

	N = 80
Central V, mean (SD)	7.9 (4.0)
Inner V, mean (SD)	16.1 (3.4)
Outer V, mean (SD)	17.5 (1.8)
Full V, mean (SD)	17.0 (2.0)
Central P, mean (SD)	17.7 (9.1)
Inner P, mean (SD)	39.1 (6.8)
Outer P, mean (SD)	43.2 (4.5)
Full P, mean (SD)	41.5 (5.0)
FAZ area, mean (SD)	0.3 (0.1)
FAZ perimeter, mean (SD)	2.2 (0.6)
FAZ circumference, mean (SD)	0.7 (0.1)
SIV, mean (SD)	18.4 (18.1)
NIV, mean (SD)	18.0 (15.1)
IIV, mean (SD)	16.4 (2.9)
TIV, mean (SD)	16.5 (2.9)
SOV, mean (SD)	17.4 (2.0)
NOV, mean (SD)	19.0 (1.8)
IOV, mean (SD)	17.4 (2.1)
TOV, mean (SD)	16.1 (2.4)
SIP, mean (SD)	39.6 (7.5)
NIP, mean (SD)	38.0 (8.7)
IIP, mean (SD)	39.3 (7.5)
TIP, mean (SD)	38.8 (7.5)
SOP, mean (SD)	43.2 (5.3)
NOP, mean (SD)	46.4 (4.7)
IOP, mean (SD)	46.9 (38.0)
TOP, mean (SD)	39.4 (6.5)

V: vascular density; P: perfusion; FAZ: Foveal avascular zone; SIV: vessel density of superior inner sector; NIV vessel density of nasal inner sector; IIV vessel density of inferior inner sector; TIV: vessel density of inner temporal sector; SOV: vessel density of superior outer sector; NOV: vessel density of nasal outer sector; IOV: vessel density of inferior outer sector; TOV: vessel density of temporal outer sector; SIP: perfusion of superior inner sector; NIP: perfusion of nasal inner sector; IIP: perfusion of inferior inner sector; TIP: perfusion of temporal inner sector; SOP: perfusion of superior outer sector; NOP: perfusion of nasal outer sector; IOP: perfusion of inferior outer sector; TOP: temporal outer perfusion; SD: standard deviation.

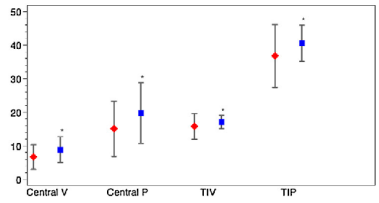

Fig. 3. Macular OCTA parameters according to D-dimer levels. The figure depicts the mean and standard deviation of the OCTA parameters that showed significant differences between COVID-19 patients with Dimer D levels ≥ 500 ng/ml (red) and those with levels < 500 ng/ml (blue). The OCTA parameters represented are central vascular density (Central V), central perfusion (Central P), vascular density in the temporal inner sector (TIV) and vascular perfusion in the temporal inner sector (TIP). *p value < 0.05.

thromboembolic complications and blood coagulation disorders.[3] D-Dimer elevation, a marker of thrombus formation, has been reported to be one of the most frequent laboratory findings noted in hospitalized COVID-19 patients, representing an independent predictor of hospital mortality.[13,14] Our results revealed that patients with an increased DD, and therefore, a prothrombotic state during the acute phase of the infection, showed lower macular vessel density and perfusion in certain OCTA parameters after the infection has resolved.

Retinal vasculature may be affected by SARS-CoV-2 infection through different mechanisms. The fact that the retina is one of the highest energy-consuming tissues in the body, turns it into a particularly vulnerable tissue to ischemia.[15] Retinal vascular disorders has been demonstrated to be associated with systemic coagulopathies and prothrombotic states.[16,17] In addition, the retinal vasculature endothelium may be also susceptible to the direct damage by SARS-CoV-2.[18] Since there is evidence of the presence of angiotensin-converting enzyme 2 (ACE2), a functional receptor of SARS-CoV-2, in arterial and venous endothelial cells, vascular damage at this level seems feasible.[19] Therefore, the retinal vasculature might be altered by a conjunction of events that include thromboembolisms, a hypercoagulable state, hypoxia and endothelial cell dysfunction.

Since the eye offers a readily accessible window to evaluate subclinical and clinical retinal vasculature changes, it seems meaningful to evaluate and quantify through OCTA the retinal involvement related to COVID-19. OCTA has demonstrated to be very useful in the study of other systemic diseases that associate retinal vascular disorders, such as diabetes mellitus and arterial hypertension.[20,21] Donati et al. found that the retinal vascular density showed pathological modifications between healthy subjects and hypertensive patients.[6] These findings suggested that OCTA may identify pathological markers of an early vascular damage, providing a rationale for retinal vasculature evaluation by OCTA in patients with recently resolved COVID-19.

Our results show a decrease in macular vessel density in patients with higher D-dimer values. This suggests that SARS-CoV-2 may produce subclinical changes at the level of the retinal vasculature, probably related to the potential prothrombotic action and the hypercoagulable state induced by SARS-CoV-2 infection. Since the retinal microvasculature shares many morphological and physiological properties with the vasculature of other organs such as the brain, the kidney and the coronary arteries, we could hypothesize that these results could be reflected in other vital organs.[22,23]

COVID-19 patients have shown laboratory abnormalities in other parameters despite D-Dimer, such as a decrease in lymphocytes and platelet count, increased CRP and elevated fibrinogen and ferritin concentrations.[24] However, we have not found a correlation between OCTA parameters and these abnormalities. The fact that inflammatory laboratory parameters such as CRP had not shown differences in retinal vessel density and perfusion, and one coagulation parameter, D-dimer, had, raises the hypothesis that the underlying physiopathology of these retinal findings may have a thrombotic origin. Moreover, our study found no relationship between the clinical severity of the disease and the retinal vascular changes. Therefore, the microvascular involvement related to COVID-19 might depend on the procoagulant state of the patient rather than on the severity of the illness. This would support the fact that subclinical thromboembolic events may occur in patients with increased D-dimer despite having a mild COVID-19.

The present study has several limitations. Critically ill patients are not fully represented in our sample, because some have died and others remained admitted in the hospital. Since severe COVID-19 is more prone to thromboembolic events and coagulopathies, evaluating these patients could yield more significant data on changes at the retinal vasculature level. Furthermore, patients over 65 years old were excluded from this study. Firstly, because these patients present a higher incidence of ophthalmic disease that could condition the results, and secondly, because they represent population at risk that should avoid going to hospitals unless they require emergency care during this critical situation. Thus, the differences found in a sample with younger patients may be less evident and therefore require a larger group to find differences. On the other hand, our study only evaluated OCTA parameters in the superficial capillary plexus due to the fact that the available device only provides quantitative data of the superficial plexus. Consequently, quantitative analyses of the intermediate and deep plexus could yield additional information in this regard. Finally, the assessment was

performed 4 weeks after being attended in the ED, in order to follow mandatory isolation. It would have been interesting to have evaluated the patients in an earlier stage of the disease. However, the extraordinary pandemic situation, the limited resources and the restrictive access of infected patients to common areas did not allow it.

In conclusion, a growing body of evidence exhibits that retinal vascular changes may represent a novel biomarker, reflecting the sequels of an underlying microvascular disease. Our results show that OCTA can detect microvascular changes not otherwise noted on dilated clinical examination in COVID-19 patients. The fact that elevated D-Dimer values during SARS-CoV-2 infection are associated with a decrease of retinal vascular density and perfusion raises the possibility of further vascular involvement in other organs besides the eye. More robust studies are warranted to fully elucidate the significance of these findings.

Authors' contribution

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole.

Ethics approval

The study was approved by the Clinical Research Ethics Committee of HCSC and was conducted in accordance with the Helsinki Declaration.

Consent to participate

Written informed consent was obtained from all patients.

Consent for publication

All authors have given their approval for this version to be published.

Availability of data and code availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Funding

No funding or sponsorship was received for this study or publication of this article.

Conflict of interest

All authors declare that they have no conflict of interest.

Acknowledgements

We wish to thank investigators of COVID-19_URG-HCSC Register: Juan González del Castillo, Adrián Valls Carbó, Enrique del Toro, Eduardo Cardassay, Gabriel Cozar López, María del Mar Suárez-Cadenas, Pablo Jerez Fernández, Beatriz Angós, Cristina Díaz del Arco, Esther Rodríguez Adrada, María Teresa Montalvo Moraleda, Carolina Espejo Paeres, Amanda López Picado, Carmen Martínez Valero, Juande D. Miranda, David Chaparro, Miguel Ángel García Briñón, José Luis Fernández Rueda, José Mª Leal Pozuelo, José Luis Fernández Rueda, Víctor Hernández Martín-Romo.

References

1. World Health Organization. Pneumonia of unknown cause – China; 5 January 2020. https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/ [accessed 1.10.20].
2. Ciceri F, Beretta L, Scandroglio AM, Colombo S, Landoni G, Ruggeri A, et al. Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome (MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. Crit Care Resusc J Australas Acad Crit Care Med. 2020.
3. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol. 2020, http://dx.doi.org/10.1016/S2352-3026(20)30145-9.
4. Kashani AH, Chen C-L, Gahm JK, Zheng F, Richter GM, Rosenfeld PJ, et al. Optical coherence tomography angiography: a comprehensive review of current methods and clinical applications. Prog Retin Eye Res. 2017;60:66–100, http://dx.doi.org/10.1016/j.preteyeres.2017.07.002.
5. Conigliaro P, Cesareo M, Chimenti MS, Triggianese P, Canofari C, Aloe G, et al. Evaluation of retinal microvascular density in patients affected by systemic lupus erythematosus: an optical coherence tomography angiography study. Ann Rheum Dis. 2019;78:287–9, http://dx.doi.org/10.1136/annrheumdis-2018-214235.
6. Donati S, Maresca AM, Cattaneo J, Grossi A, Mazzola M, Caprani SM, et al. Optical coherence tomography angiography and arterial hypertension: a role in identifying subclinical microvascular damage? Eur J Ophthalmol. 2019, http://dx.doi.org/10.1177/1120672119880390, 112067211988039.
7. Lee J, Rosen R. Optical coherence tomography angiography in diabetes. Curr Diab Rep. 2016;16:123, http://dx.doi.org/10.1177/1120672119899901.
8. Pirani, Pelliccioni, De Turris, Rosati, Franceschi, Cesari, et al. The eye as a window to systemic infectious diseases: old enemies, new imaging. J Clin Med. 2019;8:1392, http://dx.doi.org/10.3390/jcm8091392.
9. World Health Organization. Criteria for realising COVID-19 patients from isolation; 17 June 2020. https://www.who.int/publications/i/item/criteria-for-releasing-covid-19-patients-from-isolation [accessed 1.07.20].
10. World Health Organization. WHO R&D Blueprint Novel Coronavirus COVID-19 Therapeutic Trial Synopsis; 18 February 2020. https://www.who.int/blueprint/priority-diseases/key-action/COVID-19_Treatment_Trial_Design_Master_Protocol_synopsis_Final_18022020.pdf [accessed 1.06.20].
11. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708–20, http://dx.doi.org/10.1056/NEJMoa2002032.
12. Roberts CM, Bugler JR, Melchor R, Hetzel MR, Spiro SG. Value of pulse oximetry in screening for long-term oxygen therapy requirement. Eur Respir J. 1993;6:559–62.
13. Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1 MERS-CoV and lessons from the past. J Clin Virol. 2020;127:104362, http://dx.doi.org/10.1016/j.jcv.2020.104362.
14. Zhang L, Yan X, Fan Q, Liu H, Liu X, Liu Z, et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. J Thromb Haemost. 2020, http://dx.doi.org/10.1111/jth.14859, jth.14859.
15. Lee C-W, Cheng H-C, Chang F-C, Wang A-G. Optical coherence tomography angiography evaluation of retinal microvasculature before and after carotid angioplasty and stenting. Sci Rep. 2019;9:14755, http://dx.doi.org/10.1038/s41598-019-51382-8.
16. Hayreh SS, Zimmerman B, McCarthy MJ, Podhajsky P. Systemic diseases associated with various types of retinal vein occlusion. Am J Ophthalmol. 2001;131:61–77, http://dx.doi.org/10.1016/S0002-9394(00)00709-1.
17. Biswas JKRR, Pal B, Gondhale HP, Kharel (Sitaula) R. Long-term outcomes of a large cohort of patients with Eales' disease. Ocul Immunol Inflamm. 2018;26:870–6, http://dx.doi.org/10.1080/09273948.2017.1298817.
18. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395:1417–8, http://dx.doi.org/10.1016/S0140-6736(20)30937-5.
19. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004;203:631–7, http://dx.doi.org/10.1002/path.1570.
20. Khadamy J, Aghdam K, Falavarjani K. An update on optical coherence tomography angiography in diabetic retinopathy. J Ophthalmic Vis Res. 2018;13:487, http://dx.doi.org/10.4103/jovr.jovr_57_18.
21. Guemes-Villahoz N, Burgos-Blasco B, Donate-Lopez J, Garcia-Feijoo J. Retinal findings in COVID-19 patients with diabetes mellitus. Diabetes Res Clin Pract. 2020:108395, http://dx.doi.org/10.1016/j.diabres.2020.108395.
22. Flammer J, Konieczka K, Bruno RM, Virdis A, Flammer AJ, Taddei S. The eye and the heart. Eur Heart J. 2013;34:1270–8, http://dx.doi.org/10.1093/eurheartj/eht023.
23. Patton N, Aslam T, Macgillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J Anat. 2005;206:319–48, http://dx.doi.org/10.1111/j.1469-7580.2005.00395.x.
24. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med. 2020, http://dx.doi.org/10.1515/cclm-2020-0198.

DISCUSIÓN

6 DISCUSIÓN

La infección por el SARS-CoV-2, causante de la COVID-19, ha mostrado manifestaciones clínicas en prácticamente todos los órganos, incluido el ojo. A pesar de que la principal vía de transmisión de la infección son las gotas respiratorias y aerosoles, la presencia de ARN del SARS-CoV-2 en lágrima y exudado conjuntival, pone de relevancia el papel de la superficie ocular como posible vía de transmisión de la infección. En nuestro trabajo se detectó ARN de SARS-CoV-2 mediante PCR de lágrima y exudado conjuntival en dos pacientes ingresados con COVID-19 (2/36). Trabajos previos habían sugerido que la presencia de ARN de SARS-CoV-2 en lágrima estaba condicionada por la presencia de conjuntivitis asociada a la enfermedad.[107,149] Xia y colaboradores analizaron las muestras de lágrima y secreción conjuntival de 30 pacientes con neumonía COVID-19. De ellos, el único paciente que reveló resultados positivos en la RT-PCR presentaba conjuntivitis.[149] Otro informe de la provincia de Hubei, China, encontró resultados positivos para el SARS-CoV-2 en la RT-PCR de hisopos conjuntivales y nasofaríngeos de dos pacientes con conjuntivitis.[107] A la luz de estos resultados, se sugirió inicialmente que el valor diagnóstico de la prueba podría ser mayor en los pacientes COVID-19 que presentaban conjuntivitis que en los que no la presentaban. De acuerdo con estos hallazgos, en nuestro trabajo se analizaron dos grupos de pacientes, 18 pacientes COVID-19 que manifestaron conjuntivitis durante el ingreso hospitalario y 18 pacientes COVID-19 que no asociaron conjuntivitis. Se encontró la misma tasa de resultados positivos en el grupo con conjuntivitis (5.5%) que en el grupo sin conjuntivitis (5.5%). Estos resultados sugieren que la detección del SARS-CoV-2 en fluidos oculares no está condicionada por la presencia de conjuntivitis asociada a COVID-19. Todos los pacientes incluidos en nuestro trabajo presentaron resultados positivos en la PCR de exudado nasofaríngeo. Sin embargo, sólo se detectó el virus en la muestra ocular de dos de ellos.

Por tanto, el empleo de muestras de secreciones oculares para la detección de ARN de SARS-CoV-2, parece tener un valor diagnóstico limitado debido a la baja tasa de resultados positivos encontrados. Nuestro trabajo encontró una tasa de positivos en la PCR de muestra ocular similar a la obtenida por otros autores.[79,150,151] Por el momento, no se conoce con exactitud el motivo de la baja tasa de detección del virus en fluidos oculares. La sensibilidad de la RT-PCR para este tipo de muestras y el momento de la recogida de la muestra se han propuesto como posibles factores responsables. La media de días transcurridos desde el inicio de los síntomas de COVID-19 hasta la recogida de las muestras en nuestro estudio fueron de 10 días (rango, 2-19). Estos datos son similares a los encontrados por Sonmez y colaboradores, quienes encontraron una tasa de positividad de SARS-CoV-2 en lágrima del 2.5% durante la fase inicial de la infección (primeros 7 días).[151] Dado que la mayor parte de las lágrimas son drenadas a través del sistema nasolagrimal a la cavidad nasal, es posible que el virus pase rápidamente de la superficie ocular al sistema respiratorio, y por tanto su presencia en la superficie ocular sería por un tiempo limitado. Aunque también se ha publicado el caso de una mujer de 65 años con conjuntivitis, en la que se detectó ARN del SARS-CoV-2 en exudado conjuntival durante 18 días consecutivos (de los días 3 a 21 de la enfermedad), y posteriormente, 5 días después de que se volviera indetectable, el virus se detectó de nuevo en la muestra del día 27.[152] Estos hallazgos sugieren que algunos pacientes podrían mostrar una replicación sostenida del virus en la conjuntiva. La presencia del ARN del SARS-CoV-2 en las secreciones oculares podría explicarse por la inoculación del virus en el ojo. Esta transmisión podría producirse, bien por inoculación accidental de partículas víricas de las manos del paciente, o bien por contacto ocular directo con gotas respiratorias o fómites contaminados, como ocurre en otros tipos de conjuntivitis víricas como la conjuntivitis por Adenovirus. El método de recogida y la cantidad de muestra

obtenida con cada uno de ellos son otros de los factores que podrían explicar la baja detección del virus en muestras de la superficie ocular. Un estudio obtuvo las muestras empleando tanto un test de Schirmer como un hisopo conjuntival.[153] Los resultados de este estudio revelaron una capacidad de detección de ARN viral en las muestras de hisopo conjuntival (14.7%) mayor que con tira de papel de Schirmer (9.3%). En base a estos resultados, los autores sugieren el empleo de hisopo conjuntival como la técnica de elección para la recogida de la muestra. No obstante, se precisa estandarización en el protocolo de recogida de las muestras, lo que podría aportar datos más sólidos sobre la persistencia del virus en el ojo. La prevalencia notificada de detección de ARN viral en lágrima y exudado conjuntival varía entre diferentes estudios entre el 0% y el 7%,[152–154] con tasas de positividad más elevadas en los pacientes con COVID-19 grave (24%).[153] Arora y colaboradores obtuvieron muestras de exudado conjuntival de 75 pacientes con COVID-19 moderado-grave, y obtuvieron resultados positivos en 18 pacientes (24%).[153] Lo que sugiere que la posibilidad de transmisión del virus a través de la superficie ocular es significativamente mayor en los pacientes con COVID-19 de moderada a grave. Curiosamente, los dos pacientes que arrojaron resultado positivo en la PCR de lágrima fueron varones de avanzada edad (90 y 92 años) con COVID-19 grave. Además, el Ct fue relativamente elevado (Ct 25 en ambos pacientes), lo que apoyaría dicha hipótesis. Hubiera sido interesante recoger muestras conjuntivales consecutivas de los dos pacientes con PCR positiva para comprender mejor la dinámica viral y cuantificar el Ct a lo largo del proceso de la enfermedad. Sin embargo, debido a los limitados reactivos y kits de RT-PCR durante la situación de pandemia en la que se realizó este trabajo, sólo se pudo recoger una única muestra por paciente. Finalmente, puesto que la prueba diagnóstica RT-PCR no ofrece una sensibilidad del 100%, en algunos casos, los resultados negativos de la prueba podrían suponer falsos negativos y, por tanto, no excluir la presencia del

virus. Un método para aumentar esta sensibilidad hubiera sido recoger varias muestras por paciente en diferentes fases de la enfermedad. Aunque, como hemos comentado anteriormente, en el momento álgido de la crisis sanitaria, debido a los reactivos limitados y a la priorización de recursos, sólo se pudo obtener una única muestra por paciente. De hecho, las muestras de los pacientes incluidos en nuestro trabajo fueron recogidas en marzo y abril de 2020, almacenadas en el laboratorio de microbiología del HCSC, y posteriormente, analizadas en mayo de 2020, cuando hubo mayor disponibilidad de recursos.

El segundo estudio incluido en el compendio de publicaciones de esta Tesis estudió la frecuencia y características clínicas de la conjuntivitis asociada a la COVID-19. Nuestro trabajo reveló una frecuencia de conjuntivitis del 11.6% en un grupo de 301 pacientes hospitalizados con COVID-19.[109] Algunas publicaciones han observado mayor prevalencia de conjuntivitis a mayor gravedad de la enfermedad, llegando a encontrar conjuntivitis en un 28% de los pacientes con COVID-19 grave, en comparación con un 9.3% en los de presentación clínica leve a moderada.[155] En este sentido, aunque nuestro trabajo no encontró relación con la gravedad, el análisis de subgrupos reveló que la conjuntivitis fue más frecuente en los varones clasificados clínicamente con COVID-19 moderada y en las mujeres con COVID-19 leve. Estos resultados podrían estar en relacionados con las diferencias encontradas en cuanto a género en la gravedad del COVID-19.[156,157] Diferentes estudios han encontrado diferencias en la respuesta inmunitaria al SARS-CoV-2 entre hombres y mujeres, así como mayor gravedad y mortalidad en hombres que en mujeres.[158,159] Un estudio multicéntrico que incluyó a 308.010 adultos con COVID-19 reveló que los varones tienen una mayor tasa de intubación respiratoria, una mayor duración de la estancia hospitalaria y una mayor tasa de mortalidad que las mujeres.[160] A pesar de estas diferencias en la clínica y el pronóstico

de la COVID-19, nuestro estudio no mostró diferencias en la presentación clínica de la conjuntivitis entre hombres y mujeres. Además, es interesante destacar que de un matrimonio hospitalizado con COVID-19 que dormía en la misma habitación en casa y presentaba la misma gravedad clínica, sólo la mujer presentó conjuntivitis. Esto sugiere que quizás la aparición de la conjuntivitis podría depender de las características del huésped o del mecanismo de inoculación. La mediana del intervalo de tiempo entre la aparición de los síntomas de COVID-19 y la aparición de la conjuntivitis fue de 6 días (p25-p75: 2-13), cifras similares a las encontradas en las revisiones de otros estudios.[110] Esta conjuntivitis se presenta habitualmente como una conjuntivitis folicular inespecífica que puede afectar a uno o ambos ojos. Sus características clínicas incluyen hiperemia conjuntival, quemosis, epífora y secreción.[107,109] La evolución natural de la conjuntivitis se ha descrito como el de una conjuntivitis habitualmente leve, de curso autolimitado, que se resuelve sin complicaciones a corto plazo en un intervalo de tiempo medio de 3 días.[109,110] Es llamativa la ausencia de petequias y hemorragias subconjuntivales en nuestra muestra, a pesar de que diferentes artículos informan de las complicaciones vasculares y trombóticas en diferentes órganos asociadas al virus.[161] Tampoco encontramos ninguna complicación asociada, como infiltrados y membranas o pseudomembranas, al igual que la mayoría de los estudios publicados hasta la fecha.[107,109,121,155] No obstante, Navel y colaboradores publicaron un caso clínico de conjuntivitis hemorrágica con pseudomembranas en un paciente de 63 años ingresado en la unidad de cuidados intensivos (UCI) por COVID-19. El análisis de las muestras de este paciente no identificó ninguna etiología bacteriana ni viral (incluido SARS-CoV-2) que pudiera explicar esta conjuntivitis y los signos clínicos comenzaron 17 días después del inicio de síntomas COVID-19, tras 11 días de ingreso en el entorno de la UCI. Esto hace pensar que posiblemente el SARS-CoV-2 no fuera el único organismo en probable

relación con esta conjuntivitis, de la que, hasta la fecha, ha sido el único caso publicado.[162] El diagnóstico diferencial de la conjuntivitis por SARS-CoV-2 incluye otras conjuntivitis víricas, como la conjuntivitis por Adenovirus, la cual comparte mecanismos de transmisión e incluso, presenta una clínica en muchos aspectos similar a la de la COVID-19. Según datos publicados por un estudio realizado en España, la frecuencia de la conjuntivitis viral experimentó una reducción relativa importante (48,5%) durante la segunda mitad del año 2020, lo que se relaciona con las medidas higiénico-sociales impuestas debido a la pandemia.[163] Otro estudio similar encontró que, entre el 19 de marzo y 30 de abril de 2019, se atendieron un total de 1.139 pacientes en Urgencias oftalmológicas, de las cuales 162 (14,2%) tuvieron un diagnóstico clínico de conjuntivitis. En 2020, en ese mismo Hospital y periodo de tiempo, se atendieron 280 pacientes, 19 de ellos (6,8%) diagnosticados de conjuntivitis. De modo que no sólo se observó una importante reducción en las Urgencias oftalmológicas atendidas, sino además una disminución significativa en la incidencia de casos de conjuntivitis por adenovirus en 2020 en comparación con 2019 ($p = 0,001$).[164] De lo que se concluye que, en periodos de alta incidencia COVID-19, ante un paciente con conjuntivitis, se debe tener en cuenta a la conjuntivitis COVID-19 dentro del diagnóstico diferencial. En nuestro trabajo, el 54,29% de las conjuntivitis estudiadas fueron unilaterales. Por otro lado, la conjuntivitis adenovírica presenta una mayor tendencia a la bilateralidad.[165] A pesar de esto, la conjuntivitis asociada a la COVID-19 también se puede presentar de manera bilateral, lo que complica en muchos casos el diagnóstico diferencial. La evolución natural de las conjuntivitis de la muestra estudiada fue una resolución espontánea que no precisó tratamiento específico más allá de las medidas de aislamiento de contacto, limpieza de las secreciones con suero salino fisiológico y lubricación con lágrima artificial. Los pacientes presentaron mejoría progresiva desde el comienzo de los

síntomas, a diferencia de la conjuntivitis de origen por Adenovirus, en la que solemos encontrar un empeoramiento durante los primeros días y pueden durar más de 14 días. La demostración de la asociación directa entre la conjuntivitis y la infección por el SARS-CoV-2 en ausencia de la confirmación diagnóstica con PCR de lágrima y secreción conjuntival es difícil de demostrar. Sin embargo, a la luz de los resultados obtenidos por nuestro grupo de trabajo y expuestos previamente, dada la baja tasa de positivos encontrados mediante RT-PCR de lágrima y secreciones conjuntivales, un resultado negativo tampoco descartaría por completo la presencia del virus. Por este motivo, y debido a las medidas restrictivas de acceso a los pacientes con COVID-19 y recursos limitados, no se realizó RT-PCR de muestras oculares en estos pacientes. Si bien es cierto, ante un paciente con conjuntivitis, especialmente en periodo de alta incidencia de la infección, se debe valorar la realización de una PCR de exudado nasofaríngeo en caso de signos o síntomas sospechosos de la infección. Basándonos en nuestros hallazgos, estimamos que la prevalencia real de conjuntivitis podría estar infraestimada, en parte porque muchos casos leves o muy leves pueden haber pasado desapercibidos tanto por el personal sanitario como por los propios pacientes, ya que en aquel momento se priorizó los cuadros clínicos más graves. Además, dado que se trata de un estudio transversal, los pacientes que presentaron conjuntivitis antes del ingreso no fueron evaluados por los investigadores, sino que fueron evaluados por los médicos de atención primaria. La exclusión de los pacientes con deterioro cognitivo o síndrome confusional sugiere que también podría haber un cambio en la prevalencia real. Al tratarse de un hospital terciario en el centro de Madrid que cubre un área sanitaria con una población envejecida, fue necesario excluir a un elevado número de pacientes por deterioro cognitivo, estado confusional y condiciones críticas, con el objeto obtener datos más fiables. Este trabajo fue el primer estudio que describió las características clínicas de la conjuntivitis en una

amplia muestra de pacientes con COVID-19. La frecuencia de conjuntivitis encontrada en nuestra muestra (11,6%), nos permite inferir que aproximadamente 1 de cada 10 pacientes afectados por la COVID-19 pueden presentar síntomas de conjuntivitis asociados a la enfermedad. Nuestras observaciones pueden ayudar a los oftalmólogos y a otros médicos a identificar a posibles pacientes con COVID-19 que presenten sintomatología compatible con conjuntivitis. En cualquier caso, se precisan estudios prospectivos y ensayos clínicos que analicen la posibilidad de un manejo terapéutico específico en el caso de la conjuntivitis asociada a la COVID-19, así como la posibilidad del empleo de antisépticos tópicos que, empleados a nivel ocular, pudieran disminuir la transmisibilidad de la infección. En este sentido se ha valorado el empleo de la povidona yodada a nivel ocular como método preventivo adyuvante en las exploraciones oftalmológicas en la era COVID-19.[166] La povidona yodada es un antiséptico ampliamente utilizado en oftalmología previo a procedimientos quirúrgicos e inyecciones intravítreas. [167,168] Diversos estudios han confirmado que la povidona yodada inactiva numerosos virus respiratorios comunes, incluido el SARS-CoV-2.[169,170] De hecho, por esta actividad virucida, diversos autores han recomendado el empleo de enjuagues bucales con actividad antimicrobiana previos a procedimientos dentales con el fin de reducir la carga viral durante los procedimientos orales.[169,171] En cuanto a la conjuntivitis, la povidona yodada también ha sido empleada a diferentes dosis y con resultados variables en el tratamiento de conjuntivitis virales, como la conjuntivitis adnovírica.[172–174] Un estudio realizado in vitro, evaluó la actividad del empleo de 2 gotas diarias de aceite ozonizado en gel liposomado en las células Vero E6 infectadas con una cepa primaria de SARS-CoV-2.[175] Los autores encontraron una reducción en la replicación viral de 70 veces a las 72 horas, en comparación con las no tratadas. Otro estudio in vitro realizado en Italia, evaluó la actividad antiviral de cuatro colirios disponibles

comercialmente en el país: Lipozoneye (Ozodrop, FBVision, Ascoli Piceno, Italia), Vitamina E TPGS (Dropsept, IROMED group s.r.l., Roma, Italia), hipoclorito de sodio (Septavis, MEDIVIS, Catania, Italia), yodo (Iodim, MEDIVIS, Catania, Italia), contra el SARS-CoV-2.[176] En conjunto, los hallazgos mostraron que estos colirios pueden actuar sobre la adhesión del virus a la célula huésped. Sin embargo, la evidencia in vivo disponible hasta el momento es insuficiente para recomendar el empleo generalizado de estos tratamientos para la conjuntivitis asociada a la COVID-19.

En la presente Tesis también se estudió la afectación retiniana de la COVID-19, mediante la realización de fondo de ojo, OCT y OCTA.[135,177,178] La microangiopatía retiniana asociada a la COVID-19 se puede presentar con hemorragias retinianas, exudados algodonosos, tortuosidad vascular y dilatación venosa.[137–139] Estos hallazgos han sido publicados tanto en pacientes hospitalizados con COVID-19 grave, como en pacientes asintomáticos.[136] Por lo que su posible asociación con comorbilidades o el empeoramiento y desestabilización de enfermedades previas durante la COVID-19 también ha sido discutida por distintos autores.[136,137,139] Nuestro grupo realizó fondo de ojo a un total de 90 pacientes COVID-19 con diagnóstico confirmado de la infección mediante PCR de exudado nasofaríngeo. No se detectó la presencia de hemorragias retinianas, exudados, ni otros signos compatibles con una posible microangiopatía retiniana asociada a la COVID-19 en estos pacientes. Tampoco ninguno de los pacientes refirió disminución de la visión u otros síntomas oculares relevantes durante la enfermedad, ni hasta el momento de la evaluación. El trabajo publicado por Zapata y colaboradores tampoco detectó lesiones funduscópicas en la exploración realizada a 69 pacientes recuperados COVID-19, incluido pacientes con COVID-19 severo, lo que cuestiona si realmente existe una relación directa entre los hallazgos clínicos encontrados en la retina por otros autores y la infección por SARS-CoV-2.[179] No obstante, el número

de pacientes reclutados en nuestro trabajo es limitado. Además, se excluyeron de este estudio los sujetos mayores de 65 años. En primer lugar, porque estos pacientes presentan una mayor incidencia de enfermedades oftalmológicas que pudieran condicionar los resultados, y, en segundo lugar, porque representan una población de riesgo que debía evitar acudir a los hospitales por motivos distintos a requerir una atención de urgencia durante el periodo crítico de la pandemia. Así, las diferencias encontradas en una muestra con pacientes más jóvenes pueden ser menos evidentes y, por tanto, podría requerir un grupo más numeroso para encontrar diferencias. Por otra parte, la exploración oftalmológica se realizó de media a las 4 semanas[178] y 12 semanas[177] tras ser atendidos en el servicio de Urgencias del HCSC, por lo que la posible afectación retiniana durante la fase aguda de la infección, podría no estar presente clínicamente en una exploración posterior. Riotto y colaboradores realizaron un estudio prospectivo y observacional en 172 pacientes hospitalizados consecutivamente con COVID-19, en el que los pacientes se sometieron a una retinografía de campo amplio en el mismo momento del ingreso hospitalario.[180] Se detectó la presencia de exudados algodonosos y/o hemorragias retinianas en 19/172 pacientes (11%). Todos estos hallazgos habían desaparecido en la revisión realizada a los 3 meses. Hubiera sido interesante evaluar a los pacientes incluidos en nuestro trabajo durante la fase aguda de la infección, aunque el escenario y la logística hospitalaria impidieron dicha evaluación durante el pico de la pandemia. Este trabajo también realizó exploración OCT en pacientes COVID-19, y lo comparó con controles sanos.[135] El grupo de control se formó con controles históricos sanos reclutados para una base de datos normativa en 2018. Se decidió el empleo de controles históricos, tanto por la dificultad de obtener controles sanos en el momento del estudio, como por la imposibilidad de descartar con certeza antecedentes de infección previa. Recordemos que la especificidad y sensibilidad de las pruebas diagnósticas no es del 100% y se ha

reportado una alta prevalencia de pacientes asintomáticos.[87] Los pacientes recuperados de COVID-19 mostraron un aumento del grosor global de la CFNR peripapilar, así como en múltiples sectores, comparado con los controles sanos. Además, el grosor del CCG estaba aumentado en el grupo COVID-19 comparado con el grupo control. Por el contrario, el grosor de la CFNR estaba disminuido a nivel macular en pacientes recuperados de la COVID-19. En múltiples enfermedades neurodegenerativas se ha descrito la afectación de las capas internas de la retina mediante OCT. En el caso de la etiología de la enfermedad de Parkinson, la hipótesis de Braak propone la posibilidad de una enfermedad infecciosa que se extiende desde el sistema nervioso entérico hacia el cerebro, a través del nervio vago.[181] Curiosamente, la fase preclínica del Parkinson puede presentar síntomas olfativos y gastrointestinales, de forma similar a la COVID-19. En la OCT de los pacientes con Parkinson, el grosor global de la retina, así como el grosor de la CFNR peripapilar, la CCG y la capa plexiforme interna está disminuido como resultado del daño nervioso.[182,183] También en este sentido, se ha publicado que otra enfermedad de origen nervioso, la esclerosis múltiple, podría ser desencadenada por un agente infeccioso, siendo un virus la causa más probable.[184] Se proponen dos vías teóricas a través de las cuales el SARS-COV-2 podría invadir el SNC, la vía hematógena y la vía neuronal retrógrada.[185] Por lo tanto, los cambios observados en nuestra serie podrían deberse a un daño agudo, y estos, podrían o bien volver a su grosor basal, o desarrollar atrofia a largo plazo.

Nuestro grupo de trabajo, realizó un análisis independiente de una serie de casos que incluyó sólo cinco pacientes COVID-19, de los cuáles se disponía de pruebas de OCT basales (previas a la pandemia), debido a que se trataban de pacientes en seguimiento en nuestro servicio de oftalmología.[186] En este trabajo comparamos los datos de los parámetros de OCT peripapilar obtenidos mediante OCT antes y después de la COVID-

19. Los resultados de este análisis mostraron también un aumento del grosor de la CFNR global (media: 4,3 μm) en la exploración realizada 4 semanas tras la COVID-19 con respecto a los exámenes anteriores. Estos resultados apoyan la teoría de una posible inflamación a nivel nervioso detectable de manera no invasiva mediante OCT. En un trabajo publicado por Singh y colaboradores, donde se inocularon cepas neurotrópicas del virus de la hepatitis de ratón en ratones (MHV), se observó que el mejor método para inducir la neuroinflamación que conduce a la inflamación del nervio óptico, es la inoculación intracraneal, siendo superior a la inoculación del virus vía intranasal.[187] Estos resultados sugieren que los efectos neurotrópicos del SARS-CoV-2 en el nervio óptico, podrían ser el resultado de una infección directa neuronal con transporte axonal del virus desde el cerebro hacia el nervio óptico. De hecho, se han observado cambios en el grosor de la CFNR peripapilar en las neuritis ópticas secundarias a otras enfermedades infecciosas.[188,189] Por lo que nuestros resultados parecen congruentes con la respuesta neuroinflamatoria asociada al SARS-CoV-2.

Otro hallazgo interesante extraído de nuestro trabajo es que los pacientes que habían presentado anosmia y ageusia durante la fase aguda de la infección presentaban un aumento significativo del grosor de la CFNR peripapilar y del grosor de la CCG macular, en comparación con los pacientes COVID-19 que no habían referido estos síntomas. Sin embargo, no se encontraron diferencias en los parámetros de OCT en cuanto a otra sintomatología neurológica como la cefalea y los mareos. La alteración del olfato y el gusto es una sintomatología fuertemente asociada con la COVID-19.[190] Se han observado cambios en el bulbo olfatorio mediante resonancia magnética cerebral en pacientes COVID-19 con anosmia.[191,192] Algunos autores consideran la posibilidad de que el SARS-CoV-2 acceda al SNC a través de un invasión transmucosa olfativa del virus de forma similar a otros virus neurotrópicos.[193] Por tanto, los cambios observados cuando se

estratifica a los pacientes COVID-19 por la presentación de anosmia o ageusia, apoyan la idea de que estos síntomas son clave en el neurotropismo viral por la retina y nervio óptico.

Finalmente, en nuestro trabajo no encontramos los cambios hiperreflectivos en las imágenes de OCT descritos en otros trabajos.[122,131] La vinculación de estos hallazgos con la COVID-19 ha sido cuestionada por otros autores, quienes sostienen que estos cambios representan secciones oblicuas y transversales de los vasos sanguíneos de la retina.[194] Estas placas hiperreflectivas en la OCT son similares a las encontradas en la maculopatía media paracentral aguda (PAMM).[195] De hecho, se han publicado casos de neurorretinopatía macular aguda (NMA) y de su variante PAMM en posible relación con la COVID-19.[196,197] Ambas entidades se han vinculado con una isquemia del plexo capilar de la retina, fisiopatología que podría ser congruente con la afectación vascular que produce la infección por SARS-CoV-2. Llama la atención el aumento de casos de NMA que se ha observado durante la pandemia de COVID-19, tanto asociados a la propia infección SARS-CoV-2, como a la vacunación del COVID-19.[198] Se ha notificado la presencia de sintomatología respiratoria tipo gripal o fiebre en casi el 50% de los pacientes sin COVID-19 con AMN, lo que también apunta a una infección vírica como factor potencial que desempeña un papel en estas patologías. Por tanto, es posible que estas alteraciones microvasculares afecten a la circulación retiniana en sus plexos superficiales y profundos, generando áreas de señales hiperreflectantes en las capas internas de la retina.

En los trabajos de esta Tesis también se evaluó la afectación de la vascularización de la retina en pacientes COVID-19 mediante OCTA.[177,178] Nuestro estudio reveló diferencias cuantitativas en la microcirculación de la retina entre los pacientes con COVID-19 y los controles sanos. Los pacientes con antecedentes de infección por SARS-CoV-2

presentaban una densidad vascular (DV) y una densidad de perfusión (PD) reducida comparada con controles sanos 12 semanas después de la fase aguda de la infección. Estos resultados aportan información relevante sobre la afectación microvascular de la infección por SARS-CoV-2 y las posibles secuelas vasculares de la COVID-19. Además, en este mismo trabajo se comparó los resultados de los parámetros de OCTA de pacientes COVID-19 que sufrieron eventos trombóticos asociados a la enfermedad, con aquellos de pacientes COVID-19 que no presentaron complicaciones trombóticas asociadas. Los resultados no mostraron diferencias en los parámetros de OCTA al considerar los eventos trombóticos, lo que puede indicar que la afectación microvascular del SARS-CoV-2 a nivel de la retina no está condicionada por la presencia de eventos trombóticos a otros niveles. Los episodios trombóticos constituyen una de las complicaciones más importantes de la infección por SARS-CoV-2.[148,199] Se ha descrito una alta incidencia de complicaciones tromboembólicas en pacientes con COVID-19, más aún en pacientes críticos, reportando incidencias variables que van del 11,5 al 27%.[147,200] A pesar de que la trombosis pulmonar representa la manifestación trombótica más frecuente asociada a la infección,[200] también se han descrito complicaciones trombóticas en otros órganos, incluido el ojo.[201] El mecanismo a través del cual el SARS-CoV-2 puede afectar a la circulación sanguínea retiniana se desconoce por el momento. La retina podría verse afectada tanto por el daño tisular directo del SARS-CoV-2 y su inmunogenicidad como por el estado de hipercoagulabilidad asociado a la enfermedad.[202] Estudios recientes sugieren que la COVID-19 es en realidad una enfermedad vascular que afecta al endotelio a través del receptor ACE2, el cual se expresa en diversos órganos del cuerpo, incluyendo las células endoteliales de la vascularización retiniana.[136] Recordemos que ACE-2 y TMPRSS son las principales moléculas que intervienen en la entrada celular del SARS-CoV-2, y ambas han sido detectadas en la retina de estudios post mortem.[203] Se ha

observado que la ECA-2 se expresa en múltiples células neurorretinianas no vasculares, incluyendo la capa de células ganglionares de la retina, la capa plexiforme interna, la capa nuclear interna y los segmentos externos de los fotorreceptores, así como en las células endoteliales de la retina. También se ha observado la expresión de TMPRSS2 en múltiples células neuronales de la retina, en células vasculares y perivasculares, y en la glía de Müller.[203] En contraste con estos hallazgos, un estudio reciente no detectó la expresión genética de ACE2 y TMPRSS2 en la retina, aunque sí detectó otros posibles genes intervinientes en la entrada celular del SARS-CoV-2 como FURIN, CTSB y CTSL.[204] Este mismo trabajo detectó la expresión de ACE2 y TMPRSS2 en las células vasculares de la coroides, a pesar de no haberlo encontrado en la retina. Teniendo en cuenta el conocimiento actual sobre la entrada del virus en la célula huésped, los autores de este estudio sugieren una baja susceptibilidad del segmento posterior al SARS-CoV-2 con un posible punto débil en los vasos sanguíneos de la retina, los cuales podrían desempeñar un papel causal putativo en los hallazgos retinianos encontrados en pacientes COVID-19. Casagrande y colaboradores detectaron ARN de SARS-CoV-2 en las biopsias de la retina realizadas a 3 pacientes fallecidos por COVID-19, hecho que algunos autores atribuyen a una posible contaminación de las muestras.[204,205] También se han detectado posibles partículas virales mediante inmunofluorescencia y microscopía electrónica de transmisión,[203] por lo que el mecanismo de entrada del SARS-CoV-2 en la retina está aún por determinar. Además de la microangiopatía retiniana asociada a la COVID-19, se han observado oclusiones vasculares retinianas, tanto arteriales como venosas, en pacientes con COVID-19.[202,206–209] Estos hallazgos parecen estar relacionados con el estado de hipercoagulabilidad e inflamación inducida por el SARS-CoV-2.[202] Asimismo, estas anomalías no sólo se producen en pacientes con COVID-19 grave o con factores de riesgo, también se dan en pacientes jóvenes, sin factores de riesgo conocidos y/o

enfermedad COVID-19 leve. Ninguno de los pacientes incluidos en nuestro trabajo presentó signos compatibles con una obstrucción arterial o venosa retiniana asociada a la COVID-19.

Un estudio reciente evaluó la incidencia de las oclusiones arteriales y venosas retinianas durante el periodo comprendido entre el 1 de marzo y el 31 de diciembre de 2020.[117] Como se esperaba, el número total de nuevos pacientes que fueron atendidos en las clínicas de retina disminuyó drásticamente durante los primeros meses de la pandemia. Sin embargo, la reducción en el número de nuevos pacientes diagnosticados con obstrucción de la arteria central de la retina (OACR), obstrucción de la vena central de la retina (OVCR) y rama venosa retiniana no fue tan drástica como la reducción en el número de nuevos pacientes totales. Según los autores, esto parece indicar que los pacientes con patologías más urgentes y visualmente significativas seguían acudiendo a las urgencias oftalmológicas con más frecuencia que aquellos con patologías no urgentes durante los primeros meses de la pandemia. De ahí que este estudio concluye que el aumento en el porcentaje de nuevos pacientes diagnosticados con CRAO, CRVO y BRAO, en comparación con todos los pacientes, puede haber llevado a algunos oftalmólogos a tener la impresión de que la incidencia de estas patologías ha aumentado durante la pandemia de COVID-19. En realidad, si se tiene en cuenta todo el periodo estudiado (marzo-diciembre de 2020), el porcentaje de nuevos casos de obstrucción arterial y venosa retiniana se mantuvo estable durante la mayor parte del periodo COVID-19.[208] Puesto que el examen oftalmológico en este trabajo se realizó 12 semanas después del diagnóstico de COVID-19, los posibles cambios vasculares en la retina durante la fase aguda de la infección podrían no estar presentes clínicamente en el examen posterior. La mejoría de la situación epidemiológica y los cambios en los protocolos de actuación, probablemente permitan en el futuro mayor accesibilidad a una exploración

oftalmológica detallada de los pacientes COVID-19 durante la fase aguda de infección. También se han correlacionado los datos obtenidos mediante OCTA con los parámetros clínicos y de laboratorio. El Dímero D (DD) es un producto de degradación de fibrina que constituye un marcador de formación de trombos. La elevación del DD es uno de los hallazgos de laboratorio más frecuentes que se observan en los pacientes hospitalizados por COVID-19 y representa un predictor independiente de la mortalidad hospitalaria.[210,211] Por ello, su asociación con cambios vasculares de la retina pueden representar un nuevo biomarcador que refleje la afectación de una enfermedad microvascular subyacente en otros órganos. Nuestros resultados muestran una disminución de la DV a nivel macular en los pacientes con valores de dímero D >500ng/ml. Los pacientes con COVID-19 han mostrado anomalías de laboratorio en otros parámetros además del Dímero-D, como una disminución de los linfocitos y del recuento de plaquetas, un aumento de la proteína C reactiva (CRP) y concentraciones elevadas de fibrinógeno y ferritina. Sin embargo, en nuestro trabajo no se observó una correlación entre los parámetros de OCTA y estas anomalías. El hecho de que los parámetros inflamatorios de laboratorio, como la CRP, no hayan mostrado diferencias en los datos de OCTA, y un parámetro de coagulación, el dímero D, sí lo haya hecho, plantea la hipótesis de que la fisiopatología subyacente de estos hallazgos retinianos pueda tener un origen trombótico. Esto sugiere que el SARS-CoV-2 puede producir cambios subclínicos a nivel de la vasculatura de la retina, probablemente relacionados con la potencial acción protrombótica y el estado de hipercoagulabilidad inducido por la infección del SARS-CoV-2.[146,161,210] Dado que la microvasculatura de la retina comparte muchas propiedades morfológicas y fisiológicas con la vasculatura de otros órganos, como el cerebro, el riñón y las arterias coronarias, podríamos plantear la hipótesis de que estos resultados podrían reflejarse en otros órganos vitales. Además, nuestro estudio no encontró relación entre la

gravedad clínica de la enfermedad y los cambios vasculares retinianos. Por tanto, la afectación microvascular relacionada con la COVID-19 podría depender del estado procoagulante del paciente más que de la gravedad de la enfermedad. Esto apoyaría el hecho de que los eventos tromboembólicos subclínicos pueden ocurrir en pacientes con aumento del DD a pesar de tener una COVID-19 leve.

No obstante, los pacientes críticos no están totalmente representados en nuestra muestra, bien porque algunos fallecieron, o porque permanecían ingresados en el hospital. Dado que los pacientes con COVID-19 grave tienen mayor riesgo de sufrir eventos tromboembólicos y coagulopatías,[82] la evaluación de estos pacientes podría arrojar datos más significativos sobre los cambios a nivel de la vasculatura retiniana. Por otro lado, nuestro estudio sólo evaluó los parámetros de la OCTA en el plexo capilar superficial (PCS) debido a que el dispositivo disponible sólo proporciona datos cuantitativos de dicho plexo. En consecuencia, los análisis cuantitativos del plexo capilar profundo (PCP) podrían aportar información adicional al respecto. Otras patologías vasculares como la retinopatía diabética o las oclusiones vasculares retinianas han mostrado una mayor afectación vascular en el PCP mediante OCTA.[212,213] Cennamo y colaboradores encontraron una mayor alteración de los parámetros de OCTA en el PCP que en el PCS en pacientes COVID-19.[214] La estructura vascular del PCP se caracteriza por una fina red capilar, lo que lo convierte en un plexo más vulnerable a eventos trombóticos que el mayor calibre vascular del PCS. No obstante, estudios prospectivos con un mayor número de pacientes podrían aportar más datos sobre el impacto a largo plazo de la infección por SARS-CoV-2, así como responder a los todavía numerosos interrogantes acerca de la repercusión ocular de la COVID-19. Dado que el ojo ofrece una ventana fácilmente accesible para evaluar los cambios subclínicos y clínicos de la vasculatura de la retina,

parece razonable evaluar y cuantificar mediante OCTA la afectación de la retina relacionada con la COVID-19.

CONCLUSIONES

7 CONCLUSIONES

Las conclusiones por objetivos de este trabajo son:

1. El ARN del SARS-CoV-2 puede detectarse mediante RT-PCR en muestra de lágrima y exudado conjuntival de pacientes COVID-19 con y sin conjuntivitis.

2. La prueba RT-PCR de muestra de lágrima y exudado conjuntival no es un método diagnóstico útil para el diagnóstico de infección por SARS-CoV-2 debido a la baja tasa de resultados positivos obtenidos.

3. La conjuntivitis es una manifestación oftalmológica frecuente de la COVID-19 que se presenta como una conjuntivitis vírica, inespecífica y autolimitada, que se resuelve sin complicaciones en la muestra estudiada.

4. Se detectó conjuntivitis en aproximadamente 1 de cada 10 pacientes hospitalizados con COVID-19.

5. Los pacientes recuperados de COVID-19 presentan alteraciones en el grosor de la capa de fibras nerviosas de la retina y de células ganglionares detectables mediante Tomografía de coherencia óptica.

6. La densidad vascular y de la densidad de perfusión está disminuida en los pacientes recuperados de COVID-19 en comparación con los controles sanos mediante angiografía tomografía de coherencia óptica (OCTA).

7. No se encontraron diferencias significativas en los parámetros vasculares retinianos detectables mediante de OCTA entre los pacientes COVID-19 que sufrieron eventos trombóticos asociados a la enfermedad y aquellos que no.

8. Existe una disminución de la densidad vascular en los pacientes COVID-19 que presentaron valores de dímero D elevados durante la enfermedad.

BIBLIOGRAFÍA

8 BIBLIOGRAFÍA

1. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol*. 2020;5(4):536-544. doi:10.1038/s41564-020-0695-z
2. World Health Organization. WHO Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020. https://www.who.int/director-general/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020
3. Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. *Acta Biomed*. 2020;91(1):157-160. doi:10.23750/abm.v91i1.9397
4. World Health Organization. No Title. https://covid19.who.int/region/euro/country/es
5. White-Dzuro G, Gibson LE, Zazzeron L, et al. Multisystem effects of COVID-19: a concise review for practitioners. *Postgrad Med*. 2021;133(1):20-27. doi:10.1080/00325481.2020.1823094
6. Guan W jie, Ni Z yi, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720. doi:10.1056/NEJMoa2002032
7. Nasiri N, Sharifi H, Bazrafshan A, Noori A, Karamouzian M, Sharifi A. Ocular Manifestations of COVID-19: A Systematic Review and Meta-analysis. *J Ophthalmic Vis Res*. Published online January 20, 2021. doi:10.18502/jovr.v16i1.8256
8. Wang MY, Zhao R, Gao LJ, Gao XF, Wang DP, Cao JM. SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. *Front Cell Infect Microbiol*. 2020;10. doi:10.3389/fcimb.2020.587269
9. Anderson RM, Fraser C, Ghani AC, et al. Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic. *Philos Trans R Soc Lond B Biol Sci*. 2004;359(1447):1091-1105. doi:10.1098/rstb.2004.1490
10. Ksiazek TG, Erdman D, Goldsmith CS, et al. A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. *N Engl J Med*. 2003;348(20):1953-1966. doi:10.1056/NEJMoa030781
11. Hui DS. Tracking the transmission and evolution of MERS-CoV. *Lancet*. 2013;382(9909):1962-1964. doi:10.1016/S0140-6736(13)61955-8
12. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395(10224):565-574. doi:10.1016/S0140-6736(20)30251-8
13. Joint WHO-China Study. *WHO-Convened Global Study of Origins of SARS-CoV-2: China Part*.
14. World Health Organization. Modes of transmission of the COVID-19 virus. https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations
15. Hawks SA, Prussin AJ, Kuchinsky SC, Pan J, Marr LC, Duggal NK. Infectious SARS-CoV-2 Is Emitted in Aerosol Particles. Lednicky JA, ed. *MBio*. 2021;12(5). doi:10.1128/mBio.02527-21
16. Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. *Lancet*. 2021;397(10285):1603-1605. doi:10.1016/S0140-6736(21)00869-2
17. William C. Hinds. *Aerosol Technology: Properties, Behavior, and Measurement*

of Airborne Particles.; 1999.
18. Shiu EYC, Leung NHL, Cowling BJ. Controversy around airborne versus droplet transmission of respiratory viruses: implication for infection prevention. *Curr Opin Infect Dis*. 2019;32(4):372-379. doi:10.1097/QCO.0000000000000563
19. Gralton J, Tovey E, McLaws ML, Rawlinson WD. The role of particle size in aerosolised pathogen transmission: A review. *J Infect*. 2011;62(1):1-13. doi:10.1016/j.jinf.2010.11.010
20. Siegel JD, Rhinehart E, Jackson M, Chiarello L. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. *Am J Infect Control*. 2007;35(10):S65-S164. doi:10.1016/j.ajic.2007.10.007
21. Tang JW, Bahnfleth WP, Bluyssen PM, et al. Dismantling myths on the airborne transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J Hosp Infect*. 2021;110:89-96. doi:10.1016/j.jhin.2020.12.022
22. Prather KA, Marr LC, Schooley RT, McDiarmid MA, Wilson ME, Milton DK. Airborne transmission of SARS-CoV-2. Sills J, ed. *Science (80-).* 2020;370(6514):303.2-304. doi:10.1126/science.abf0521
23. Chau NVV, Hong NTT, Ngoc NM, et al. Superspreading Event of SARS-CoV-2 Infection at a Bar, Ho Chi Minh City, Vietnam. *Emerg Infect Dis*. 2021;27(1). doi:10.3201/eid2701.203480
24. Hamner L, Dubbel P, Capron I, et al. High SARS-CoV-2 Attack Rate Following Exposure at a Choir Practice - Skagit County, Washington, March 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(19):606-610. doi:10.15585/mmwr.mm6919e6
25. Miller SL, Nazaroff WW, Jimenez JL, et al. Transmission of SARS-CoV-2 by inhalation of respiratory aerosol in the Skagit Valley Chorale superspreading event. *Indoor Air*. 2021;31(2):314-323. doi:10.1111/ina.12751
26. van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N Engl J Med*. 2020;382(16):1564-1567. doi:10.1056/NEJMc2004973
27. Tang S, Mao Y, Jones RM, et al. Aerosol transmission of SARS-CoV-2? Evidence, prevention and control. *Environ Int*. 2020;144:106039. doi:10.1016/j.envint.2020.106039
28. Guo ZD, Wang ZY, Zhang SF, et al. Aerosol and Surface Distribution of Severe Acute Respiratory Syndrome Coronavirus 2 in Hospital Wards, Wuhan, China, 2020. *Emerg Infect Dis*. 2020;26(7):1583-1591. doi:10.3201/eid2607.200885
29. Centers for disease control and prevention. SARS-CoV-2 Transmission. https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/sars-cov-2-transmission.html#anchor_1619805150492
30. Centers for disease control and prevention. Science Brief: SARS-CoV-2 and Surface (Fomite) Transmission for Indoor Community Environments. https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/surface-transmission.html#ref1
31. Chia PY, Coleman KK, Tan YK, et al. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. *Nat Commun*. 2020;11(1):2800. doi:10.1038/s41467-020-16670-2
32. Cai J, Sun W, Huang J, Gamber M, Wu J, He G. Indirect Virus Transmission in Cluster of COVID-19 Cases, Wenzhou, China, 2020. *Emerg Infect Dis*. 2020;26(6):1343-1345. doi:10.3201/eid2606.200412
33. Goldman E. Exaggerated risk of transmission of COVID-19 by fomites. *Lancet Infect Dis*. 2020;20(8):892-893. doi:10.1016/S1473-3099(20)30561-2

34. Dowell SF, Simmerman JM, Erdman DD, et al. Severe Acute Respiratory Syndrome Coronavirus on Hospital Surfaces. *Clin Infect Dis*. 2004;39(5):652-657. doi:10.1086/422652
35. Deniz M, Tezer H. Vertical transmission of SARS CoV-2: a systematic review. *J Matern Neonatal Med*. Published online July 21, 2020:1-8. doi:10.1080/14767058.2020.1793322
36. Schwartz DA, Thomas KM. Characterizing COVID-19 maternal-fetal transmission and placental infection using comprehensive molecular pathology. *EBioMedicine*. 2020;60:102983. doi:10.1016/j.ebiom.2020.102983
37. Kunjumon B, Wachtel E V., Lumba R, et al. Breast Milk and Breastfeeding of Infants Born to SARS-CoV-2 Positive Mothers: A Prospective Observational Cohort Study. *Am J Perinatol*. 2021;38(11):1209-1216. doi:10.1055/s-0041-1731451
38. Centeno-Tablante E, Medina-Rivera M, Finkelstein JL, et al. Transmission of SARS-CoV-2 through breast milk and breastfeeding: a living systematic review. *Ann N Y Acad Sci*. 2021;1484(1):32-54. doi:10.1111/nyas.14477
39. Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal–oral transmission of SARS-CoV-2. *Nat Rev Gastroenterol Hepatol*. 2021;18(4):269-283. doi:10.1038/s41575-021-00416-6
40. Kang M, Wei J, Yuan J, et al. Probable Evidence of Fecal Aerosol Transmission of SARS-CoV-2 in a High-Rise Building. *Ann Intern Med*. 2020;173(12):974-980. doi:10.7326/M20-0928
41. Arora R, Goel R, Kumar S, et al. Evaluation of SARS-CoV-2 in Tears of Patients with Moderate to Severe COVID-19. *Ophthalmology*. 2021;128(4):494-503. doi:10.1016/j.ophtha.2020.08.029
42. Sopp NM, Sharda V. An Eye on COVID-19: A Meta-analysis of Positive Conjunctival Reverse Transcriptase-Polymerase Chain Reaction and SARS-CoV-2 Conjunctivitis Prevalence. *Optom Vis Sci*. 2021;98(5):429-436. doi:10.1097/OPX.0000000000001687
43. Lu CW, Liu XF, Jia ZF. 2019-nCoV transmission through the ocular surface must not be ignored. *Lancet (London, England)*. 2020;395(10224):e39. doi:10.1016/S0140-6736(20)30313-5
44. Raboud J, Shigayeva A, McGeer A, et al. Risk Factors for SARS Transmission from Patients Requiring Intubation: A Multicentre Investigation in Toronto, Canada. Montgomery JM, ed. *PLoS One*. 2010;5(5):e10717. doi:10.1371/journal.pone.0010717
45. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. *Nat Rev Mol Cell Biol*. Published online October 5, 2021. doi:10.1038/s41580-021-00418-x
46. Baughn LB, Sharma N, Elhaik E, Sekulic A, Bryce AH, Fonseca R. Targeting TMPRSS2 in SARS-CoV-2 Infection. *Mayo Clin Proc*. 2020;95(9):1989-1999. doi:10.1016/j.mayocp.2020.06.018
47. Yao Y, Ma D, Xu Y, et al. Hydroxychloroquine treatment on SARS-CoV-2 receptor ACE2, TMPRSS2 and NRP1 expression in human primary pterygium and conjunctival cells. *Exp Eye Res*. 2022;214:108864. doi:10.1016/j.exer.2021.108864
48. Mencucci R, Favuzza E, Becatti M, et al. Co-expression of the SARS-CoV-2 entry receptors ACE2 and TMPRSS2 in healthy human conjunctiva. *Exp Eye Res*. 2021;205:108527. doi:10.1016/j.exer.2021.108527
49. Zhou L, Xu Z, Guerra J, et al. Expression of the SARS-CoV-2 Receptor ACE2 in

Human Retina and Diabetes—Implications for Retinopathy. *Investig Opthalmology Vis Sci*. 2021;62(7):6. doi:10.1167/iovs.62.7.6
50. Wang K, Chen W, Zhang Z, et al. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct Target Ther*. 2020;5(1):283. doi:10.1038/s41392-020-00426-x
51. Määttä M, Tervahartiala T, Kaarniranta K, et al. Immunolocalization of EMMPRIN (Cd147) in the Human Eye and Detection of Soluble Form of EMMPRIN in Ocular Fluids. *Curr Eye Res*. 2006;31(11):917-924. doi:10.1080/02713680600932290
52. Chen X, Yu H, Mei T, et al. SARS-CoV-2 on the ocular surface: is it truly a novel transmission route? *Br J Ophthalmol*. Published online August 11, 2020:bjophthalmol-2020-316263. doi:10.1136/bjophthalmol-2020-316263
53. Li D, Jin M, Bao P, Zhao W, Zhang S. Clinical Characteristics and Results of Semen Tests Among Men With Coronavirus Disease 2019. *JAMA Netw Open*. 2020;3(5):e208292. doi:10.1001/jamanetworkopen.2020.8292
54. Gupta P, Choudhary A, Gopal G, et al. Detection of SARS-CoV2 virus using the real-time reverse transcriptase polymerase chain reaction in semen and seminal plasma from men with active COVID-19 infection – A pilot study. *Indian J Urol*. 2021;37(4):331. doi:10.4103/iju.iju_117_21
55. Chang L, Zhao L, Gong H, Wang L, Wang L. Severe Acute Respiratory Syndrome Coronavirus 2 RNA Detected in Blood Donations. *Emerg Infect Dis*. 2020;26(7):1631-1633. doi:10.3201/eid2607.200839
56. Hogan CA, Stevens BA, Sahoo MK, et al. High Frequency of SARS-CoV-2 RNAemia and Association With Severe Disease. *Clin Infect Dis*. 2021;72(9):e291-e295. doi:10.1093/cid/ciaa1054
57. McAloon C, Collins Á, Hunt K, et al. Incubation period of COVID-19: a rapid systematic review and meta-analysis of observational research. *BMJ Open*. 2020;10(8):e039652. doi:10.1136/bmjopen-2020-039652
58. Nishiura H, Linton NM, Akhmetzhanov AR. Serial interval of novel coronavirus (COVID-19) infections. *Int J Infect Dis*. 2020;93:284-286. doi:10.1016/j.ijid.2020.02.060
59. Binnicker MJ. Can Testing Predict SARS-CoV-2 Infectivity? The Potential for Certain Methods To Be Surrogates for Replication-Competent Virus. Humphries RM, ed. *J Clin Microbiol*. 2021;59(11). doi:10.1128/JCM.00469-21
60. Plebani M. Persistent viral RNA shedding in COVID-19: Caution, not fear. *EBioMedicine*. 2021;64:103234. doi:10.1016/j.ebiom.2021.103234
61. Singanayagam A, Patel M, Charlett A, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *Eurosurveillance*. 2020;25(32). doi:10.2807/1560-7917.ES.2020.25.32.2001483
62. Ministerio de Sanidad. Gobierno de España. Parámetros epidemiológicos. https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/documentos/20210810_EPIDEMIOLOGIA.pdf
63. Lim JS, Cho SI, Ryu S, Pak SI. Interpretation of the Basic and Effective Reproduction Number. *J Prev Med Public Heal*. 2020;53(6):405-408. doi:10.3961/jpmph.20.288
64. Langa LS, Sallent LV, Díez SR. Interpretación de las pruebas diagnósticas de la COVID-19. *FMC - Form Médica Contin en Atención Primaria*. 2021;28(3):167-173. doi:10.1016/j.fmc.2021.01.005
65. Centers for disease control and prevention. Interim Guidelines for Collecting and

Handling of Clinical Specimens for COVID-19 Testing. Accessed January 10, 2022. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
66. Hellewell J, Russell TW, Beale R, et al. Estimating the effectiveness of routine asymptomatic PCR testing at different frequencies for the detection of SARS-CoV-2 infections. *BMC Med*. 2021;19(1):106. doi:10.1186/s12916-021-01982-x
67. La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM. Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. *Reprod Biomed Online*. 2020;41(3):483-499. doi:10.1016/j.rbmo.2020.06.001
68. Mallett S, Allen AJ, Graziadio S, et al. At what times during infection is SARS-CoV-2 detectable and no longer detectable using RT-PCR-based tests? A systematic review of individual participant data. *BMC Med*. 2020;18(1):346. doi:10.1186/s12916-020-01810-8
69. Goverment of Canada. Polymerase chain reaction (PCR) and cycle threshold (Ct) values in COVID-19 testing.
70. Han MS, Byun JH, Cho Y, Rim JH. RT-PCR for SARS-CoV-2: quantitative versus qualitative. *Lancet Infect Dis*. 2021;21(2):165. doi:10.1016/S1473-3099(20)30424-2
71. World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection. Published 2021. file:///Users/Noemi/Downloads/WHO-2019-nCoV-Antigen-Detection-2021.1-eng.pdf
72. Wang YH, Wu CC, Bai CH, et al. Evaluation of the diagnostic accuracy of COVID-19 antigen tests: A systematic review and meta-analysis. *J Chinese Med Assoc*. 2021;84(11):1028-1037. doi:10.1097/JCMA.0000000000000626
73. Nordgren J, Sharma S, Olsson H, et al. SARS-CoV-2 rapid antigen test: High sensitivity to detect infectious virus. *J Clin Virol*. 2021;140:104846. doi:10.1016/j.jcv.2021.104846
74. Dřevínek P, Hurych J, Kepka Z, et al. The sensitivity of SARS-CoV-2 antigen tests in the view of large-scale testing. *Epidemiol Mikrobiol Imunol*. 2021;70(3):156-160. http://www.ncbi.nlm.nih.gov/pubmed/34641689
75. Jegerlehner S, Suter-Riniker F, Jent P, Bittel P, Nagler M. Diagnostic accuracy of a SARS-CoV-2 rapid antigen test in real-life clinical settings. *Int J Infect Dis*. 2021;109:118-122. doi:10.1016/j.ijid.2021.07.010
76. Brihn A, Chang J, OYong K, et al. Diagnostic Performance of an Antigen Test with RT-PCR for the Detection of SARS-CoV-2 in a Hospital Setting — Los Angeles County, California, June–August 2020. *MMWR Morb Mortal Wkly Rep*. 2021;70(19):702-706. doi:10.15585/mmwr.mm7019a3
77. Xia J, Tong J, Liu M, Shen Y, Guo D. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *J Med Virol*. 2020;92(6):589-594. doi:10.1002/jmv.25725
78. Seah IYJ, Anderson DE, Kang AEZ, et al. Assessing Viral Shedding and Infectivity of Tears in Coronavirus Disease 2019 (COVID-19) Patients. *Ophthalmology*. 2020;127(7):977-979. doi:10.1016/j.ophtha.2020.03.026
79. Zhou Y, Duan C, Zeng Y, et al. Ocular Findings and Proportion with Conjunctival SARS-COV-2 in COVID-19 Patients. *Ophthalmology*. 2020;127(7):982-983. doi:10.1016/j.ophtha.2020.04.028
80. Güemes-Villahoz N, Burgos-Blasco B, Vilela AA, et al. Detecting SARS-CoV-2 RNA in conjunctival secretions: is it a valuable diagnostic method of COVID-19? *J Med Virol*. Published online June 24, 2020. doi:10.1002/jmv.26219

81. Mehta OP, Bhandari P, Raut A, Kacimi SEO, Huy NT. Coronavirus Disease (COVID-19): Comprehensive Review of Clinical Presentation. *Front Public Heal*. 2021;8. doi:10.3389/fpubh.2020.582932
82. Fu L, Wang B, Yuan T, et al. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: A systematic review and meta-analysis. *J Infect*. 2020;80(6):656-665. doi:10.1016/j.jinf.2020.03.041
83. Casas-Rojo JM, Antón-Santos JM, Millán-Núñez-Cortés J, et al. Características clínicas de los pacientes hospitalizados con COVID-19 en España: resultados del Registro SEMI-COVID-19. *Rev Clínica Española*. 2020;220(8):480-494. doi:10.1016/j.rce.2020.07.003
84. European Centre for Disease Prevention and Control. https://www.ecdc.europa.eu/en/covid-19/latest-evidence/clinical.
85. Li J, Huang DQ, Zou B, et al. Epidemiology of COVID-19: A systematic review and meta-analysis of clinical characteristics, risk factors, and outcomes. *J Med Virol*. 2021;93(3):1449-1458. doi:10.1002/jmv.26424
86. Chen C, Zhu C, Yan D, et al. The epidemiological and radiographical characteristics of asymptomatic infections with the novel coronavirus (COVID-19): A systematic review and meta-analysis. *Int J Infect Dis*. 2021;104:458-464. doi:10.1016/j.ijid.2021.01.017
87. Ma Q, Liu J, Liu Q, et al. Global Percentage of Asymptomatic SARS-CoV-2 Infections Among the Tested Population and Individuals With Confirmed COVID-19 Diagnosis. *JAMA Netw Open*. 2021;4(12):e2137257. doi:10.1001/jamanetworkopen.2021.37257
88. Li C, Zhu Y, Qi C, et al. Estimating the Prevalence of Asymptomatic COVID-19 Cases and Their Contribution in Transmission - Using Henan Province, China, as an Example. *Front Med*. 2021;8. doi:10.3389/fmed.2021.591372
89. Al-Qahtani M, AlAli S, AbdulRahman A, Salman Alsayyad A, Otoom S, Atkin SL. The prevalence of asymptomatic and symptomatic COVID-19 in a cohort of quarantined subjects. *Int J Infect Dis*. 2021;102:285-288. doi:10.1016/j.ijid.2020.10.091
90. Oran DP, Topol EJ. Prevalence of Asymptomatic SARS-CoV-2 Infection. *Ann Intern Med*. 2020;173(5):362-367. doi:10.7326/M20-3012
91. Kariyawasam JC, Jayarajah U, Riza R, Abeysuriya V, Seneviratne SL. Gastrointestinal manifestations in COVID-19. *Trans R Soc Trop Med Hyg*. 2021;115(12):1362-1388. doi:10.1093/trstmh/trab042
92. Gupta A, Madhavan M V., Sehgal K, et al. Extrapulmonary manifestations of COVID-19. *Nat Med*. 2020;26(7):1017-1032. doi:10.1038/s41591-020-0968-3
93. Taj S, Kashif A, Arzinda Fatima S, Imran S, Lone A, Ahmed Q. Role of hematological parameters in the stratification of COVID-19 disease severity. *Ann Med Surg*. 2021;62:68-72. doi:10.1016/j.amsu.2020.12.035
94. Mina A, van Besien K, Platanias LC. Hematological manifestations of COVID-19. *Leuk Lymphoma*. 2020;61(12):2790-2798. doi:10.1080/10428194.2020.1788017
95. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
96. Whittaker A, Anson M, Harky A. Neurological Manifestations of COVID-19: A systematic review and current update. *Acta Neurol Scand*. 2020;142(1):14-22. doi:10.1111/ane.13266
97. Collantes ME V., Espiritu AI, Sy MCC, Anlacan VMM, Jamora RDG.

Neurological Manifestations in COVID-19 Infection: A Systematic Review and Meta-Analysis. *Can J Neurol Sci / J Can des Sci Neurol*. 2021;48(1):66-76. doi:10.1017/cjn.2020.146
98. Hariyanto TI, Rizki NA, Kurniawan A. Anosmia/Hyposmia is a Good Predictor of Coronavirus Disease 2019 (COVID-19) Infection: A Meta-Analysis. *Int Arch Otorhinolaryngol*. 2021;25(01):e170-e174. doi:10.1055/s-0040-1719120
99. Ahmad S, Sohail A, Shahid Chishti MA, Aemaz Ur Rehman M, Farooq H. How common are taste and smell abnormalities in COVID-19? A systematic review and meta-analysis. *J Taibah Univ Med Sci*. Published online November 2021. doi:10.1016/j.jtumed.2021.10.009
100. Lima MA, Silva MTT, Soares CN, et al. Peripheral facial nerve palsy associated with COVID-19. *J Neurovirol*. 2020;26(6):941-944. doi:10.1007/s13365-020-00912-6
101. Sheikh AB, Chourasia PK, Javed N, et al. Association of Guillain-Barre syndrome with COVID-19 infection: An updated systematic review. *J Neuroimmunol*. 2021;355:577577. doi:10.1016/j.jneuroim.2021.577577
102. Johnson KD, Harris C, Cain JK, Hummer C, Goyal H, Perisetti A. Pulmonary and Extra-Pulmonary Clinical Manifestations of COVID-19. *Front Med*. 2020;7. doi:10.3389/fmed.2020.00526
103. Mirmoeeni S, Azari Jafari A, Hashemi SZ, et al. Cardiovascular manifestations in COVID-19 patients: A systematic review and meta-analysis. *J Cardiovasc Thorac Res*. 2021;13(3):181-189. doi:10.34172/jcvtr.2021.30
104. Li B, Yang J, Zhao F, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol*. 2020;109(5):531-538. doi:10.1007/s00392-020-01626-9
105. Freeman EE, McMahon DE, Fitzgerald ME, et al. The American Academy of Dermatology COVID-19 registry: Crowdsourcing dermatology in the age of COVID-19. *J Am Acad Dermatol*. 2020;83(2):509-510. doi:10.1016/j.jaad.2020.04.045
106. Freeman EE, McMahon DE, Lipoff JB, et al. The spectrum of COVID-19–associated dermatologic manifestations: An international registry of 716 patients from 31 countries. *J Am Acad Dermatol*. 2020;83(4):1118-1129. doi:10.1016/j.jaad.2020.06.1016
107. Wu P, Duan F, Luo C, et al. Characteristics of Ocular Findings of Patients With Coronavirus Disease 2019 (COVID-19) in Hubei Province, China. *JAMA Ophthalmol*. 2020;138(5):575. doi:10.1001/jamaophthalmol.2020.1291
108. Öncül H, Öncül FY, Alakus MF, Çağlayan M, Dag U. Ocular findings in patients with coronavirus disease 2019 (COVID-19) in an outbreak hospital. *J Med Virol*. Published online August 10, 2020. doi:10.1002/jmv.26412
109. Güemes-Villahoz N, Burgos-Blasco B, García-Feijoó J, et al. Conjunctivitis in COVID-19 patients: frequency and clinical presentation. *Graefes Arch Clin Exp Ophthalmol*. Published online August 29, 2020. doi:10.1007/s00417-020-04916-0
110. Sarkar D, Soni D, Nagpal A, et al. Ocular manifestations of RT-PCR-confirmed COVID-19 cases in a large database cross-sectional study. *BMJ Open Ophthalmol*. 2021;6(1):1-8. doi:10.1136/bmjophth-2021-000775
111. Qiao C, Zhang H, He M, et al. Symptomatic COVID-19 in Eye Professionals in Wuhan, China. *Ophthalmology*. 2020;127(9):1268-1270. doi:10.1016/j.ophtha.2020.04.026
112. Modenese A, Gobba F. Increased Risk of COVID-19-Related Deaths among

General Practitioners in Italy. *Healthcare*. 2020;8(2):155. doi:10.3390/healthcare8020155
113. Breazzano MP, Shen J, Abdelhakim AH, et al. Resident physician exposure to novel coronavirus (2019-nCoV, SARS-CoV-2) within New York City during exponential phase of COVID-19 pandemic: Report of the New York City Residency Program Directors COVID-19 Research Group. *medRxiv Prepr Serv Heal Sci*. Published online April 28, 2020. doi:10.1101/2020.04.23.20074310
114. Zhong Y, Wang K, Zhu Y, et al. Ocular manifestations in COVID-19 patients: A systematic review and meta-analysis. *Travel Med Infect Dis*. 2021;44(September 2020). doi:10.1016/j.tmaid.2021.102191
115. Ocansey S, Abu EK, Abraham CH, et al. Ocular Symptoms of SARS-CoV-2: Indication of Possible Ocular Transmission or Viral Shedding. *Ocul Immunol Inflamm*. 2020;28(8):1269-1279. doi:10.1080/09273948.2020.1799035
116. Balasopoulou A, Kokkinos P, Pagoulatos D, et al. Symposium Recent advances and challenges in the management of retinoblastoma Globe - saving Treatments. *BMC Ophthalmol*. 2017;17(1):1. doi:10.4103/ijo.IJO
117. Inomata T, Kitazawa K, Kuno T, et al. Clinical and Prodromal Ocular Symptoms in Coronavirus Disease: A Systematic Review and Meta-Analysis. *Invest Ophthalmol Vis Sci*. 2020;61(10):29. doi:10.1167/iovs.61.10.29
118. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720. doi:10.1056/NEJMoa2002032
119. Loffredo L, Pacella F, Pacella E, Tiscione G, Oliva A, Violi F. Conjunctivitis and COVID-19: A meta-analysis. *J Med Virol*. Published online April 24, 2020. doi:10.1002/jmv.25938
120. Sindhuja K, Lomi N, Asif M, Tandon R. Clinical profile and prevalence of conjunctivitis in mild COVID-19 patients in a tertiary care COVID-19 hospital: A retrospective cross-sectional study. *Indian J Ophthalmol*. 2020;68(8):1546. doi:10.4103/ijo.IJO_1319_20
121. Chen L, Deng C, Chen X, et al. Ocular manifestations and clinical characteristics of 535 cases of COVID-19 in Wuhan, China: a cross-sectional study. *Acta Ophthalmol*. 2020;98(8):e951-e959. doi:10.1111/aos.14472
122. Marinho PM, Marcos AAA, Romano AC, Nascimento H, Belfort R. Retinal findings in patients with COVID-19. *Lancet*. 2020;395(10237):1610. doi:10.1016/S0140-6736(20)31014-X
123. Vavvas DG, Sarraf D, Sadda SR, et al. Concerns about the interpretation of OCT and fundus findings in COVID-19 patients in recent Lancet publication. *Eye*. 2020;34(12):2153-2154. doi:10.1038/s41433-020-1084-9
124. Venkatesh P. Seeking clarity on retinal findings in patients with COVID-19. *Lancet*. 2020;396(10254):e36. doi:10.1016/S0140-6736(20)31922-X
125. Ouyang P, Zhang X, Peng Y, Jiang B. Seeking clarity on retinal findings in patients with COVID-19. *Lancet*. 2020;396(10254):e35. doi:10.1016/S0140-6736(20)31921-8
126. DREXLER W, FUJIMOTO J. State-of-the-art retinal optical coherence tomography. *Prog Retin Eye Res*. 2008;27(1):45-88. doi:10.1016/j.preteyeres.2007.07.005
127. Medeiros FA, Zangwill LM, Bowd C, Vessani RM, Susanna R, Weinreb RN. Evaluation of retinal nerve fiber layer, optic nerve head, and macular thickness measurements for glaucoma detection using optical coherence tomography. *Am J Ophthalmol*. 2005;139(1):44-55. doi:10.1016/j.ajo.2004.08.069

128. Lamirel C, Newman NJ, Biousse V. Optical coherence tomography (OCT) in optic neuritis and multiple sclerosis. *Rev Neurol (Paris)*. 2010;166(12):978-986. doi:10.1016/j.neurol.2010.03.024
129. Chan VTT, Sun Z, Tang S, et al. Spectral-Domain OCT Measurements in Alzheimer's Disease. *Ophthalmology*. 2019;126(4):497-510. doi:10.1016/j.ophtha.2018.08.009
130. Hu J, Jolkkonen J, Zhao C. Neurotropism of SARS-CoV-2 and its neuropathological alterations: Similarities with other coronaviruses. *Neurosci Biobehav Rev*. 2020;119:184-193. doi:10.1016/j.neubiorev.2020.10.012
131. Zhou Z, Kang H, Li S, Zhao X. Understanding the neurotropic characteristics of SARS-CoV-2: from neurological manifestations of COVID-19 to potential neurotropic mechanisms. *J Neurol*. 2020;267(8):2179-2184. doi:10.1007/s00415-020-09929-7
132. Szkodny D, Wylęgała E, Sujka-Franczak P, et al. Retinal OCT Findings in Patients after COVID Infection. *J Clin Med*. 2021;10(15):3233. doi:10.3390/jcm10153233
133. Abrishami M, Daneshvar R, Emamverdian Z, Tohidinezhad F, Eslami S. Optic Nerve Head Parameters and Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Coronavirus Disease 2019. *Ocul Immunol Inflamm*. Published online February 19, 2021:1-4. doi:10.1080/09273948.2020.1850800
134. Oren B, Aksoy Aydemır G, Aydemır E, et al. Quantitative assessment of retinal changes in COVID-19 patients. *Clin Exp Optom*. 2021;104(6):717-722. doi:10.1080/08164622.2021.1916389
135. Burgos-Blasco B, Güemes-Villahoz N, Vidal-Villegas B, et al. Optic nerve and macular optical coherence tomography in recovered COVID-19 patients. *Eur J Ophthalmol*. Published online March 15, 2021:11206721211001020. doi:10.1177/11206721211001019
136. Landecho MF, Yuste JR, Gándara E, et al. COVID-19 retinal microangiopathy as an in vivo biomarker of systemic vascular disease? *J Intern Med*. 2021;289(1):116-120. doi:10.1111/joim.13156
137. Invernizzi A, Torre A, Parrulli S, et al. Retinal findings in patients with COVID-19: Results from the SERPICO-19 study. *EClinicalMedicine*. 2020;27:100550. doi:10.1016/j.eclinm.2020.100550
138. Teo KY, Invernizzi A, Staurenghi G, Cheung CMG. COVID-19-Related Retinal Micro-vasculopathy – A Review of Current Evidence. *Am J Ophthalmol*. 2022;235:98-110. doi:10.1016/j.ajo.2021.09.019
139. Pereira LA, Soares LCM, Nascimento PA, et al. Retinal findings in hospitalised patients with severe COVID-19. *Br J Ophthalmol*. Published online October 16, 2020:bjophthalmol-2020-317576. doi:10.1136/bjophthalmol-2020-317576
140. Hagag A, Gao S, Jia Y, Huang D. Optical coherence tomography angiography: Technical principles and clinical applications in ophthalmology. *Taiwan J Ophthalmol*. 2017;7(3):115. doi:10.4103/tjo.tjo_31_17
141. Patton N, Aslam T, MacGillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. *J Anat*. 2005;206(4):319-348. doi:10.1111/j.1469-7580.2005.00395.x
142. Akmaz B, Akay F, Güven YZ, Kaptan F, Demirdal T. The long-term effect of human immunodeficiency virus infection on retinal microvasculature and the ganglion cell–inner plexiform layer: an OCT angiography study. *Graefe's Arch Clin Exp Ophthalmol*. 2020;258(8):1671-1676. doi:10.1007/s00417-020-04749-x

143. Pascual-Prieto J, Burgos-Blasco B, Ávila Sánchez-Torija M, et al. Utility of optical coherence tomography angiography in detecting vascular retinal damage caused by arterial hypertension. *Eur J Ophthalmol*. 2020;30(3):579-585. doi:10.1177/1120672119831159
144. Rodman J, Ferraz M, Baran A, Zhang B. Optical coherence tomography angiography of retinal vasculature in recovered COVID-19 patients compared to age and ethnic matched controls. *Clin Exp Optom*. Published online November 9, 2021:1-6. doi:10.1080/08164622.2021.1978817
145. Zapata MÁ, Banderas García S, Sánchez-Moltalvá A, et al. Retinal microvascular abnormalities in patients after COVID-19 depending on disease severity. *Br J Ophthalmol*. Published online December 16, 2020:bjophthalmol-2020-317953. doi:10.1136/bjophthalmol-2020-317953
146. Gando S, Wada T. Thromboplasminflammation in COVID-19 Coagulopathy: Three Viewpoints for Diagnostic and Therapeutic Strategies. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.649122
147. Rali P, O'Corragain O, Oresanya L, et al. Incidence of venous thromboembolism in coronavirus disease 2019: An experience from a single large academic center. *J Vasc Surg Venous Lymphat Disord*. 2021;9(3):585-591.e2. doi:10.1016/j.jvsv.2020.09.006
148. Benito N, Filella D, Mateo J, et al. Pulmonary Thrombosis or Embolism in a Large Cohort of Hospitalized Patients With Covid-19. *Front Med*. 2020;7. doi:10.3389/fmed.2020.00557
149. Xia J, Tong J, Liu M, Shen Y, Guo D. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *J Med Virol*. 2020;92(6):589-594. doi:10.1002/jmv.25725
150. Daryabari SH, Asadollah A, Moghadam FA, Dorostkar R, Bahramifar A, Aghamollaei H. Detection of COVID-19 in tears of ICU-admitted patients with SARS-CoV-2 infection. *Int Ophthalmol*. 2022;42(3):723-727. doi:10.1007/s10792-021-01938-3
151. Sonmez A, Aydın Kurna S, Aslan FG, Kaplan FB, Açıkalın B, Eker P. SARS-COV-2 viral load in tears of patients with COVID-19 in the early symptomatic stages: comparison of two different tear sampling methods. *Int Ophthalmol*. Published online February 18, 2022. doi:10.1007/s10792-022-02243-3
152. Chen L, Liu M, Zhang Z, et al. Ocular manifestations of a hospitalised patient with confirmed 2019 novel coronavirus disease. *Br J Ophthalmol*. 2020;104(6):748-751. doi:10.1136/bjophthalmol-2020-316304
153. Arora R, Goel R, Kumar S, et al. Evaluation of SARS-CoV-2 in Tears of Patients with Moderate to Severe COVID-19. *Ophthalmology*. 2021;128(4):494-503. doi:10.1016/j.ophtha.2020.08.029
154. Karimi S, Arabi A, Shahraki T, Safi S. Detection of severe acute respiratory syndrome Coronavirus-2 in the tears of patients with Coronavirus disease 2019. *Eye*. 2020;34(7):1220-1223. doi:10.1038/s41433-020-0965-2
155. Layikh HA, Hashim ZA, Kadum AA. Conjunctivitis and other ocular findings in patients with COVID-19 infection. *Ann Saudi Med*. 2021;41(5):280-284. doi:10.5144/0256-4947.2021.280
156. Jin JM, Bai P, He W, et al. Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. *Front Public Heal*. 2020;8. doi:10.3389/fpubh.2020.00152
157. Peckham H, de Gruijter NM, Raine C, et al. Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. *Nat*

Commun. 2020;11(1):6317. doi:10.1038/s41467-020-19741-6
158. Conti P, Younes A. Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection. *J Biol Regul Homeost Agents*. 34(2):339-343. doi:10.23812/Editorial-Conti-3
159. Yi Y, Lagniton PNP, Ye S, Li E, Xu RH. COVID-19: what has been learned and to be learned about the novel coronavirus disease. *Int J Biol Sci*. 2020;16(10):1753-1766. doi:10.7150/ijbs.45134
160. Nguyen NT, Chinn J, De Ferrante M, Kirby KA, Hohmann SF, Amin A. Male gender is a predictor of higher mortality in hospitalized adults with COVID-19. den Uil C, ed. *PLoS One*. 2021;16(7):e0254066. doi:10.1371/journal.pone.0254066
161. Xiong M, Liang X, Wei Y. Changes in blood coagulation in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. *Br J Haematol*. 2020;189(6):1050-1052. doi:10.1111/bjh.16725
162. Navel V, Chiambaretta F, Dutheil F. Haemorrhagic conjunctivitis with pseudomembranous related to SARS-CoV-2. *Am J Ophthalmol Case Reports*. 2020;19:100735. doi:10.1016/j.ajoc.2020.100735
163. Conde Bachiller Y, Puente Gete B, Gil Ibáñez L, Esquivel Benito G, Asencio Duran M, Dabad Moreno J V. [COVID-19 pandemic: Impact on the rate of viral conjunctivitis]. *Arch Soc Esp Oftalmol*. 2022;97(2):63-69. doi:10.1016/j.oftal.2021.03.002
164. Reina AR, Blazquez JG, Diaz OP, et al. Epidemiological evolution of viral conjunctivitis in ophthalmology emergency departments during the Covid-19 pandemic. *Acta Ophthalmol*. 2022;100(S267). doi:10.1111/j.1755-3768.2022.137
165. Yeu E, Hauswirth S. A Review of the Differential Diagnosis of Acute Infectious Conjunctivitis: Implications for Treatment and Management. *Clin Ophthalmol*. 2020;Volume 14:805-813. doi:10.2147/OPTH.S236571
166. O'Brien TP, Pelletier J. Topical Ocular Povidone-Iodine as an Adjunctive Preventative Practice in the Era of COVID-19. *Asia-Pacific J Ophthalmol (Philadelphia, Pa)*. 2021;10(2):142-145. doi:10.1097/APO.0000000000000353
167. Levinson JD, Garfinkel RA, Berinstein DM, Flory M, Spellman FA. Timing of Povidone-Iodine Application to Reduce the Risk of Endophthalmitis after Intravitreal Injections. *Ophthalmol Retin*. 2018;2(7):654-658. doi:10.1016/j.oret.2017.06.004
168. Durand ML. ESCRS Guidelines for Prevention and Treatment of Endophthalmitis Following Cataract Surgery. *Clin Microbiol Infect*. 2013;19(3):227-234. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3638360&tool=pmcentrez&rendertype=abstract
169. Pelletier JS, Tessema B, Frank S, Westover JB, Brown SM, Capriotti JA. Efficacy of Povidone-Iodine Nasal and Oral Antiseptic Preparations Against Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2). *Ear Nose Throat J*. 2021;100(2_suppl):192S-196S. doi:10.1177/0145561320957237
170. Wang Y, Wu Y, Wang Q, et al. Virucidal effect of povidone-iodine against SARS-CoV-2 in vitro. *J Int Med Res*. 2021;49(12):3000605211063695. doi:10.1177/03000605211063695
171. Vergara-Buenaventura A, Castro-Ruiz C. Use of mouthwashes against COVID-19 in dentistry. *Br J Oral Maxillofac Surg*. 2020;58(8):924-927. doi:10.1016/j.bjoms.2020.08.016
172. Liu SH, Hawkins BS, Ng SM, et al. Topical pharmacologic interventions versus

placebo for epidemic keratoconjunctivitis. *Cochrane database Syst Rev*. 2022;3:CD013520. doi:10.1002/14651858.CD013520.pub2
173. Dang RM, Watt K, Hui A. Povidone iodine for the treatment of adenoviral conjunctivitis. *Clin Exp Optom*. 2021;104(3):308-314. doi:10.1080/08164622.2021.1877532
174. Than T, Morettin CE, Harthan JS, et al. Efficacy of a Single Administration of 5% Povidone-Iodine in the Treatment of Adenoviral Conjunctivitis. *Am J Ophthalmol*. 2021;231:28-38. doi:10.1016/j.ajo.2021.05.018
175. Rizzo S, Savastano MC, Bortolotti D, et al. COVID-19 Ocular Prophylaxis: The Potential Role of Ozonated-Oils in Liposome Eyedrop Gel. *Transl Vis Sci Technol*. 2021;10(9):7. doi:10.1167/tvst.10.9.7
176. Petrillo F, Chianese A, De Bernardo M, et al. Inhibitory Effect of Ophthalmic Solutions against SARS-CoV-2: A Preventive Action to Block the Viral Transmission? *Microorganisms*. 2021;9(8). doi:10.3390/microorganisms9081550
177. Guemes-Villahoz N, Burgos-Blasco B, Vidal-Villegas B, et al. Reduced macular vessel density in COVID-19 patients with and without associated thrombotic events using optical coherence tomography angiography. *Graefes Arch Clin Exp Ophthalmol*. Published online May 7, 2021. doi:10.1007/s00417-021-05186-0
178. Guemes-Villahoz N, Burgos-Blasco B, Vidal-Villegas B, et al. Reduced retinal vessel density in COVID-19 patients and elevated D-dimer levels during the acute phase of the infection. *Med Clin (Barc)*. Published online January 28, 2021. doi:10.1016/j.medcli.2020.12.006
179. Zapata MÁ, Banderas García S, Sánchez-Moltalvá A, et al. Retinal microvascular abnormalities in patients after COVID-19 depending on disease severity. *Br J Ophthalmol*. 2022;106(4):559-563. doi:10.1136/bjophthalmol-2020-317953
180. Riotto E, Vladimir M, Alexis M, et al. Retinal Manifestations in Patients with COVID-19 : A Prospective Cohort Study. Published online 2022.
181. Braak H, Tredici K Del, Rüb U, de Vos RA., Jansen Steur EN., Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. 2003;24(2):197-211. doi:10.1016/S0197-4580(02)00065-9
182. Aydin TS, Umit D, Nur OM, et al. Optical coherence tomography findings in Parkinson's disease. *Kaohsiung J Med Sci*. 2018;34(3):166-171. doi:10.1016/j.kjms.2017.11.006
183. Satue M, Obis J, Alarcia R, et al. Retinal and Choroidal Changes in Patients with Parkinson's Disease Detected by Swept-Source Optical Coherence Tomography. *Curr Eye Res*. 2018;43(1):109-115. doi:10.1080/02713683.2017.1370116
184. Tarlinton RE, Martynova E, Rizvanov AA, Khaiboullina S, Verma S. Role of Viruses in the Pathogenesis of Multiple Sclerosis. *Viruses*. 2020;12(6):643. doi:10.3390/v12060643
185. Li Z, Liu T, Yang N, et al. Neurological manifestations of patients with COVID-19: potential routes of SARS-CoV-2 neuroinvasion from the periphery to the brain. *Front Med*. 2020;14(5):533-541. doi:10.1007/s11684-020-0786-5
186. Burgos-Blasco B, Güemes-Villahoz N, Donate-Lopez J, Vidal-Villegas B, García-Feijóo J. Optic nerve analysis in COVID-19 patients. *J Med Virol*. Published online July 10, 2020. doi:10.1002/jmv.26290
187. Singh M, Khan RS, Dine K, Das Sarma J, Shindler KS. Intracranial Inoculation Is More Potent Than Intranasal Inoculation for Inducing Optic Neuritis in the Mouse Hepatitis Virus-Induced Model of Multiple Sclerosis. *Front Cell Infect Microbiol*. 2018;8. doi:10.3389/fcimb.2018.00311

188. Khairallah M, Kahloun R, Abroug N, et al. Infectious optic neuropathies: a clinical update. *Eye Brain*. Published online September 2015:59. doi:10.2147/EB.S69173
189. Costello F, Coupland S, Hodge W, et al. Quantifying axonal loss after optic neuritis with optical coherence tomography. *Ann Neurol*. 2006;59(6):963-969. doi:10.1002/ana.20851
190. Sudre CH, Keshet A, Graham MS, et al. Anosmia, ageusia, and other COVID-19-like symptoms in association with a positive SARS-CoV-2 test, across six national digital surveillance platforms: an observational study. *Lancet Digit Heal*. 2021;3(9):e577-e586. doi:10.1016/S2589-7500(21)00115-1
191. Strauss SB, Lantos JE, Heier LA, Shatzkes DR, Phillips CD. Olfactory Bulb Signal Abnormality in Patients with COVID-19 Who Present with Neurologic Symptoms. *Am J Neuroradiol*. 2020;41(10):1882-1887. doi:10.3174/ajnr.A6751
192. Chiu A, Fischbein N, Wintermark M, Zaharchuk G, Yun PT, Zeineh M. COVID-19-induced anosmia associated with olfactory bulb atrophy. *Neuroradiology*. 2021;63(1):147-148. doi:10.1007/s00234-020-02554-1
193. Meinhardt J, Radke J, Dittmayer C, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat Neurosci*. 2021;24(2):168-175. doi:10.1038/s41593-020-00758-5
194. Marinho PM, Nascimento H, Marcos AAA, Romano AC, Rosen RB, Belfort R. Reply to Editorial: Interpretation of OCT and fundus findings in COVID-19 patients in recent Lancet publication. *Eye*. 2021;35(12):3442-3444. doi:10.1038/s41433-020-01283-2
195. Ortiz-Egea JM, Ruiz-Medrano J, Ruiz-Moreno JM. Retinal imaging study diagnoses in COVID-19: a case report. *J Med Case Rep*. 2021;15(1):15. doi:10.1186/s13256-020-02620-5
196. Virgo J, Mohamed M. Paracentral acute middle maculopathy and acute macular neuroretinopathy following SARS-CoV-2 infection. *Eye*. 2020;34(12):2352-2353. doi:10.1038/s41433-020-1069-8
197. Michel T, Stolowy N, Gascon P et al. Acute Macular Neuroretinopathy After COVID-19 Vaccine. *J Ophthalmic Inflamm Infect*. Published online 2021.
198. Jalink MB, Bronkhorst IHG. A Sudden Rise of Patients with Acute Macular Neuroretinopathy during the COVID-19 Pandemic. *Case Rep Ophthalmol*. Published online February 14, 2022:96-103. doi:10.1159/000522080
199. Miró Ò, Llorens P, Aguirre A, et al. Association between Covid-19 and Pulmonary Embolism (AC-19-PE study). *Thromb Res*. 2020;196:322-324. doi:10.1016/j.thromres.2020.09.010
200. Rali P, O'Corragain O, Oresanya L, et al. Incidence of venous thromboembolism in coronavirus disease 2019: An experience from a single large academic center. *J Vasc surgery Venous Lymphat Disord*. 2021;9(3):585-591.e2. doi:10.1016/j.jvsv.2020.09.006
201. Fonollosa A, Hernández-Rodríguez J, Cuadros C, et al. Characterizing COVID-19-related retinal vascular occlusions. *Retina*. 2021;Publish Ah. doi:10.1097/IAE.0000000000003327
202. Acharya S, Diamond M, Anwar S, Glaser A, Tyagi P. Unique case of central retinal artery occlusion secondary to COVID-19 disease. *IDCases*. 2020;21:e00867. doi:10.1016/j.idcr.2020.e00867
203. Araujo-Silva CA, Marcos AAA, Marinho PM, et al. Presumed SARS-CoV-2 Viral Particles in the Human Retina of Patients With COVID-19. *JAMA Ophthalmol*. 2021;139(9):1015. doi:10.1001/jamaophthalmol.2021.2795

204. Künzel SE, Bürgel T, Künzel SH, et al. LOW VULNERABILITY OF THE POSTERIOR EYE SEGMENT TO SARS-COV-2 INFECTION. *Retina*. 2022;42(2):236-243. doi:10.1097/IAE.0000000000003320
205. Casagrande M, Fitzek A, Püschel K, et al. Detection of SARS-CoV-2 in Human Retinal Biopsies of Deceased COVID-19 Patients. *Ocul Immunol Inflamm*. 2020;28(5):721-725. doi:10.1080/09273948.2020.1770301
206. Gaba WH, Ahmed D, Al Nuaimi RK, Dhanhani AA, Eatamadi H. Bilateral Central Retinal Vein Occlusion in a 40-Year-Old Man with Severe Coronavirus Disease 2019 (COVID-19) Pneumonia. *Am J Case Rep*. 2020;21. doi:10.12659/AJCR.927691
207. Montesel A, Bucolo C, Mouvet V, Moret E, Eandi CM. Case Report: Central Retinal Artery Occlusion in a COVID-19 Patient. *Front Pharmacol*. 2020;11. doi:10.3389/fphar.2020.588384
208. Al-Moujahed A, Boucher N, Fernando R, et al. Incidence of Retinal Artery and Vein Occlusions During the COVID-19 Pandemic. *Ophthalmic Surgery, Lasers Imaging Retin*. 2022;53(1):22-30. doi:10.3928/23258160-20211209-01
209. Venkatesh R, Reddy NG, Agrawal S, Pereira A. COVID-19-associated central retinal vein occlusion treated with oral aspirin. *BMJ Case Rep*. 2021;14(5):e242987. doi:10.1136/bcr-2021-242987
210. Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. *J Clin Virol*. 2020;127:104362. doi:10.1016/j.jcv.2020.104362
211. Zhang L, Yan X, Fan Q, et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. *J Thromb Haemost*. 2020;18(6):1324-1329. doi:10.1111/jth.14859
212. Ashraf M, Sampani K, Clermont A, et al. Vascular Density of Deep, Intermediate and Superficial Vascular Plexuses Are Differentially Affected by Diabetic Retinopathy Severity. *Investig Opthalmology Vis Sci*. 2020;61(10):53. doi:10.1167/iovs.61.10.53
213. Coscas F, Glacet-Bernard A, Miere A, et al. Optical Coherence Tomography Angiography in Retinal Vein Occlusion: Evaluation of Superficial and Deep Capillary Plexa. *Am J Ophthalmol*. 2016;161:160-171.e2. doi:10.1016/j.ajo.2015.10.008
214. Cennamo G, Reibaldi M, Montorio D, D'Andrea L, Fallico M, Triassi M. Optical Coherence Tomography Angiography Features in Post-COVID-19 Pneumonia Patients: A Pilot Study. *Am J Ophthalmol*. 2021;227:182-190. doi:10.1016/j.ajo.2021.03.015

ANEXOS

9 ANEXOS

9.1 DIVULGACIÓN CIENTÍFICA / PUBLICACIONES

Además de los cinco artículos que constituyen el cuerpo de la Tesis, se incluyen a continuación otras publicaciones en revistas científicas relacionadas con la afectación oftalmológica de la COVID-19 publicadas por la doctoranda:

- **Güemes-Villahoz N**, Burgos-Blasco B, Perez-Garcia P, Fernández-Vigo JI, Morales-Fernandez L, Donate-Lopez J, Ramos-Amador JT, Garcia-Feijoo J. Retinal and peripapillary vessel density increase in recovered COVID-19 children by optical coherence tomography angiography. J AAPOS. 2021 Oct 20:S1091-8531(21)00552-8. doi: 10.1016/j.jaapos.2021.06.004.
- Burgos-Blasco B, **Güemes-Villahoz N**, Morales-Fernandez L, Callejas-Caballero I, Perez-Garcia P, Donate-Lopez J, Ramos-Amador JT, Garcia-Feijoo J. Retinal nerve fibre layer and ganglion cell layer changes in children who recovered from COVID-19: a cohort study. Arch Dis Child. 2021 Aug 2:archdischild-2021-321803. doi: 10.1136/archdischild-2021-321803.
- COVIDSurg Collaborative, GlobalSurg Collaborative. SARS-CoV-2 vaccination modelling for safe surgery to save lives: data from an international prospective cohort study. Br J Surg. 2021 Mar 24:znab101. doi: 10.1093/bjs/znab101.
- **Güemes-Villahoz N**, Burgos-Blasco B, Martín-Sánchez FJ, Garcia-Feijoo J. Conjuntivitis en atención primaria: posible manifestación clínica de COVID-19 [Conjunctivitis in primary care: Possible clinical presentation of COVID-19]. Aten Primaria. 2021 Mar 17;53(5):102012. doi: 10.1016/j.aprim.2021.102012.
- Burgos-Blasco B, **Güemes-Villahoz N**, Vidal-Villegas B, Garcia-Feijoo J, Donate-Lopez J, Martin-Sanchez FJ, Gonzalez-Armengol JJ, Mendez-Hernandez CD. Optic Nerve Head Vessel Density Assessment in Recovered COVID-19

Patients: A Prospective Study Using Optical Coherence Tomography Angiography. J Glaucoma. 2021 Apr 28. doi: 10.1097/IJG.0000000000001858.

- **Güemes-Villahoz N,** Burgos-Blasco B, Martín-Sánchez FJ. Protección ocular de la transmisión de SARS-CoV-2 a través de la superficie ocular en urgencias. Emergencias. 2021 Jun;33(3):248.
- Burgos-Blasco B, **Güemes-Villahoz N**, Vidal-Villegas B, Donate-Lopez J, Garcia-Feijoo J. Evaluation of retinotoxicity of COVID-19 treatment: Hydroxychloroquine and lopinavir/ritonavir. J Med Virol. 2021 Feb;93(2):644-646. doi: 10.1002/jmv.26420.
- Burgos-Blasco B, **Güemes-Villahoz N**, Donate-Lopez J, Vidal-Villegas B, García-Feijóo J. Optic nerve analysis in COVID-19 patients. J Med Virol. 2021 Jan;93(1):190-191. doi: 10.1002/jmv.26290.
- Burgos-Blasco B, **Güemes-Villahoz N**, Santiago JL, Fernandez-Vigo JI, Espino-Paisán L, Sarriá B, García-Feijoo J, Martinez-de-la-Casa JM. Hypercytokinemia in COVID-19: Tear cytokine profile in hospitalized COVID-19 patients. Exp Eye Res. 2020 Nov;200:108253. doi: 10.1016/j.exer.2020.108253.
- **Güemes-Villahoz N**, Burgos-Blasco B, Donate-Lopez J, Garcia-Feijoo J. Retinal findings in COVID-19 patients with diabetes mellitus. Diabetes Res Clin Pract. 2020 Oct;168:108395. doi: 10.1016/j.diabres.2020.108395.
- **Güemes-Villahoz N**, Burgos-Blasco B, Vidal-Villegas B, Garcia-Feijoo J, Arriola-Villalobos P, Martínez-de-la-Casa JM, Diaz-Valle D, Konstas AG. Novel Insights into the Transmission of SARS-CoV-2 Through the Ocular Surface and its Detection in Tears and Conjunctival Secretions: A Review. Adv Ther. 2020 Oct;37(10):4086-4095. doi: 10.1007/s12325-020-01442-7.

- **Güemes-Villahoz N,** Burgos-Blasco B, Arribi-Vilela A, Arriola-Villalobos P, Vidal-Villegas B, Mendez-Fernandez R, Delgado-Iribarren A, Garcia-Feijoo J. SARS-CoV-2 RNA detection in tears and conjunctival secretions of COVID-19 patients with conjunctivitis. J Infect. 2020 Sep;81(3):452-482. doi: 10.1016/j.jinf.2020.05.070.
- Burgos-Blasco B, **Güemes-Villahoz N**, Vidal-Villegas B, Martínez-de-laCasa JM, Garcia-Feijoo J, Donate-Lopez J, Martin-Sanchez FJ, Gonzalez-Armengol JJ, Mendez-Hernandez CD. One-year changes in optic nerve head parameters in recovered COVID-10 patientes. Journal of Neuro-Ophthalmology. 2022. *Articulo aceptado*

9.2 COMUNICACIONES A CONGRESOS Y REUNIONES

TITULO: Detección del ARN del SARS-CoV-2 en lágrima y exudado conjuntival: ¿es un método de diagnóstico valioso del COVID-19?

TIPO DE PARTICIPACIÓN: Comunicación oral

PUBLICACIÓN: *J Med Virol. 2021 Jan;93(1):383-388. doi: 10.1002/jmv.26219.*

CONGRESO: 2º Congreso Nacional Multidisciplinar COVID-19 de las Sociedades Científicas de España

LUGAR, FECHA: Virtual, 12 al 16 de Abril 2021

AUTORES: Noemi Güemes Villahoz, Bárbara Burgos-Blasco, Ana Arribi-Vilela, Pedro Arriola-Villalobos, CM Rico-Luna, Ricardo Cuiña-Sardiña, Alberto Delgado-Iribarren, Julián García-Feijoo.

TITULO: Manifestaciones oftalmológicas del COVID-19 en el segmento anterior. Sesión COVID-19 Y OFTALMOLOGÍA: Cómo hemos cambiado...!

TIPO DE PARTICIPACIÓN: Ponencia

PUBLICACIÓN: Graefes Arch Clin Exp Ophthalmol. 2020 Nov;258(11):2501-2507. doi: 10.1007/s00417-020-04916-0; J Med Virol. 2021 Jan;93(1):383-388. doi: 10.1002/jmv.26219

CONGRESO: 2º Congreso Nacional Multidisciplinar COVID-19 de las Sociedades Científicas de España

LUGAR, FECHA: Virtual, 12 al 16 de Abril 2021

AUTORES: Noemi Güemes Villahoz.

TITULO: Hipercitoquinemia En COVID-19: Citoquinas En Lágrima de Pacientes COVID-19

TIPO DE PARTICIPACIÓN: Poster

PUBLICACIÓN: *Exp Eye Res*. Published online September 2020:108253. doi:10.1016/j.exer.2020.108253

CONGRESO: 2º Congreso Nacional Multidisciplinar COVID-19 de las Sociedades Científicas de España

LUGAR, FECHA: Virtual, 12 al 16 de Abril 2021

AUTORES: Bárbara Burgos-Blasco, Noemí Güemes-Villahoz, Jose Luis Santiago, José Ignacio Fernández-Vigo, Laura Espino-Paisán, Beatriz Sarriá, Julián García-Feijoo J, José María Martínez-de-la-Casa.

TITULO: Reducción de la densidad vascular retiniana en pacientes COVID-19 con y sin eventos trombóticos asociados mediante angiografía de tomografía de coherencia óptica

TIPO DE PARTICIPACIÓN: Poster

PUBLICACIÓN: Graefes Arch Clin Exp Ophthalmol. 2021 Aug;259(8):2243-2249. doi: 10.1007/s00417-021-05186-0.

CONGRESO: 97 Congreso de la Sociedad Española de Oftalmología

LUGAR, FECHA: Virtual, 2 de Octubre de 2021

AUTORES: Güemes-Villahoz N, Burgos-Blasco B, Vidal-Villegas B, Donate-López J, de la Muela MH, López-Guajardo L, Martín-Sánchez FJ, García-Feijoó J.

TITULO: Hipercitoquinemia En COVID-19: Citoquinas En Lágrima de Pacientes COVID-19

TIPO DE PARTICIPACIÓN: Poster

PUBLICACIÓN: *Exp Eye Res*. Published online September 2020:108253. doi:10.1016/j.exer.2020.108253

CONGRESO: 97 Congreso de la Sociedad Española de Oftalmología

LUGAR, FECHA: Virtual, 2 de Octubre de 2021

AUTORES: Bárbara Burgos-Blasco, Noemí Güemes-Villahoz, Jose Luis Santiago, José Ignacio Fernández-Vigo, Laura Espino-Paisán, Beatriz Sarriá, Julián García-Feijoo J, José María Martínez-de-la-Casa.

TITULO: Detección del ARN del SARS-CoV-2 en lágrima y exudado conjuntival: ¿es un método de diagnóstico valioso del COVID-19?

TIPO DE PARTICIPACIÓN: Póster

PUBLICACIÓN: *J Med Virol. 2021 Jan;93(1):383-388. doi: 10.1002/jmv.26219.*

CONGRESO: 97 Congreso de la Sociedad Española de Oftalmología

LUGAR, FECHA: Virtual, 2 de Octubre de 2021

AUTORES: Noemi Güemes Villahoz, Bárbara Burgos-Blasco, Ana Arribi-Vilela, Pedro Arriola-Villalobos, CM Rico-Luna, Ricardo Cuiña-Sardiña, Alberto Delgado-Iribarren, Julián García-Feijoo.

TITULO: Aumento de la densidad vascular en niños recuperados de COVID-19 mediante angiografía por tomografía de coherencia óptica

TIPO DE PARTICIPACIÓN: Comunicación oral

PUBLICACIÓN: *J AAPOS Off Publ Am Assoc Pediatr Ophthalmol Strabismus*. 2021;25(6):325.e1-325.e6. doi:10.1016/j.jaapos.2021.06.004

CONGRESO: XXIV Congreso Sociedad Española de Retina y Vítreo (SERV)

LUGAR, FECHA: Burgos, 22 y 23 de Octubre de 2021

AUTORES: Guemes-Villahoz N, Burgos-Blasco B, Perez-Garcia P, Fernández-Vigo JI, Morales-Fernandez L, Donate-Lopez J, Ramos-Amador JT, Garcia-Feijoo J

TITULO: Patología retiniana por COVID-19

TIPO DE PARTICIPACIÓN: Ponencia

PUBLICACIÓN:*Med Clin (Barc)*. Published online January 28, 2021. doi:10.1016/j.medcli.2020.12.006; Graefes Arch Clin Exp Ophthalmol. 2021 Aug;259(8):2243-2249. doi: 10.1007/s00417-021-05186-0; *J AAPOS Off Publ Am Assoc Pediatr Ophthalmol Strabismus*. 2021;25(6):325.e1-325.e6. doi:10.1016/j.jaapos.2021.06.004.

CONGRESO: Sociedad Española de Retina y Vítreo (SERV) <40

LUGAR, FECHA: Virtual, 19 Noviembre de 2021

AUTORES: Noemi Güemes Villahoz

TITULO: Detección del ARN del SARS-CoV-2 en lágrima y exudado conjuntival: ¿es un método de diagnóstico valioso del COVID-19?

TIPO DE PARTICIPACIÓN: Póster

PUBLICACIÓN: *J Med Virol. 2021 Jan;93(1):383-388. doi: 10.1002/jmv.26219.*

CONGRESO: Reunión anual de la Sociedad oftalmológica de Madrid

LUGAR, FECHA: Madrid, 17 de Diciembre de 2021

AUTORES: Noemi Güemes Villahoz, Bárbara Burgos-Blasco, Ana Arribi-Vilela, Pedro Arriola-Villalobos, CM Rico-Luna, Ricardo Cuiña-Sardiña, Alberto Delgado-Iribarren, Julián García-Feijoó.

TITULO: Seguimiento del nervio óptico mediante tomografía de coherencia óptica y angiografía por tomografía de coherencia óptica en pacientes COVID-19

TIPO DE PARTICIPACIÓN: Póster

PUBLICACIÓN: J Glaucoma. 2021 Aug 1;30(8):711-717. doi: 10.1097/IJG.0000000000001858. PMID: 33927148; PMCID: PMC8366516.

CONGRESO: Reunión anual de la Sociedad oftalmológica de Madrid

LUGAR, FECHA: Madrid, 17 de Diciembre de 2021

AUTORES: Bárbara Burgos-Blasco, Noemi Güemes-Villahoz, Beatriz Vidal-Villegas, Julián García-Feijoo, Juan Donate-López, Francisco Javier Martín-Sánchez FJ, Juan González-Armengol, Carmen Dora Méndez-Hernández.

TITULO: Hipercitoquinemia En COVID-19: Citoquinas En Lágrima de Pacientes COVID-19

TIPO DE PARTICIPACIÓN: Póster

PUBLICACIÓN: *Exp Eye Res*. Published online September 2020:108253. doi:10.1016/j.exer.2020.108253

CONGRESO: Reunión anual de la Sociedad oftalmológica de Madrid

LUGAR, FECHA: Madrid, 17 de Diciembre de 2021

AUTORES: Bárbara Burgos-Blasco, Noemí Güemes-Villahoz, Jose Luis Santiago, José Ignacio Fernández-Vigo, Laura Espino-Paisán, Beatriz Sarriá, Julián García-Feijoo J, José María Martínez-de-la-Casa.

TITULO: Cambios en los parámetros del nervio óptico en pacientes recuperados de COVID-19 a los doce meses de seguimiento

TIPO DE PARTICIPACIÓN: Comunicación oral

PUBLICACIÓN: J Glaucoma. 2021 Aug 1;30(8):711-717. doi: 10.1097/IJG.0000000000001858. PMID: 33927148; PMCID: PMC8366516.

CONGRESO: XVI Congreso de la Sociedad Española de Glaucoma (SEG)

LUGAR, FECHA: Palma de Mallorca, 10-12 de marzo de 2022

AUTORES: Bárbara Burgos-Blasco, Noemi Güemes-Villahoz, Beatriz Vidal-Villegas, Julián García-Feijoo, Juan Donate-López, Francisco Javier Martín-Sánchez FJ, Juan González-Armengol, Carmen Dora Méndez-Hernández.

9.3 PREMIOS

Premios recibidos relacionados con la labor científica de la temática de la tesis durante la duración del doctorado.

- **3er Premio de Investigación Castroviejo-SANTEN 2020-21:**

  Güemes-Villahoz N, Burgos-Blasco B, García-Feijoó J, Sáenz-Francés F, Arriola-Villalobos P, Martinez-de-la-Casa JM, Benítez-Del-Castillo JM, Herrera de la Muela M. Conjunctivitis in COVID-19 patients: frequency and clinical presentation. Graefes Arch Clin Exp Ophthalmol. 2020 Nov;258(11):2501-2507. doi: 10.1007/s00417-020-04916-0.

- **Premio FACO ELCHE 2021:**

  Detección del ARN del SARS-CoV-2 en las secreciones conjuntivales: ¿Es un método de diagnóstico valioso de COVID-19? (Güemes-Villahoz N, Burgos-Blasco B, Arribi-Vilela A, Arriola-Villalobos P, Rico-Luna CM, Cuiña-Sardiña R, Delgado-Iribarren A, García-Feijoó J. Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19? J Med Virol. 2021 Jan;93(1):383-388. doi: 10.1002/jmv.26219.)

9.4 COPYRIGHT

A continuación, se aportan las autorizaciones del copyright de las editoriales de las revistas para el desarrollo de este proyecto de Tesis doctoral:

- **John Wiley and sons:**

  Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19? J Med Virol. 2021 Jan;93(1):383-388. doi: 10.1002/jmv.26219.

- **SPRINGER**

  Conjunctivitis in COVID-19 patients: frequency and clinical presentation. Graefes Arch Clin Exp Ophthalmol. 2020 Nov;258(11):2501-2507. doi: 10.1007/s00417-020-04916-0.

  Reduced macular vessel density in COVID-19 patients with and without associated thrombotic events using optical coherence tomography angiography. Graefes Arch Clin Exp Ophthalmol. 2021 Aug;259(8):2243-2249. doi: 10.1007/s00417-021-05186-0.

- **SAGE Publishing**

  Optic nerve and macular optical coherence tomography in recovered COVID-19 patients. Eur J Ophthalmol. 2021 Mar 15:11206721211001019. doi: 10.1177/11206721211001019.

- **ELSEVIER**

  Reduced retinal vessel density in COVID-19 patients and elevated D-dimer levels during the acute phase of the infection. Med Clin (Barc). 2021 Jan 28:S0025-7753(21)00014-2. doi: 10.1016/j.medcli.2020.12.006.

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Mar 21, 2022

This Agreement between HOSPITAL CLINICO SAN CARLOS -- NOEMI GUEMES-VILLAHOZ ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	5272921037602
License date	Mar 20, 2022
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Journal of Medical Virology
Licensed Content Title	Detecting SARS□CoV□2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID□19?
Licensed Content Author	Noemi Güemes□Villahoz, Barbara Burgos□Blasco, Ana Arribi□Vilela, et al
Licensed Content Date	Jul 6, 2020
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Mar 21, 2022

This Agreement between HOSPITAL CLINICO SAN CARLOS -- NOEMI GUEMES-VILLAHOZ ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	5273191232793
License date	Mar 20, 2022
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Graefe's Archive for Clinical and Experimental Ophthalmology
Licensed Content Title	Conjunctivitis in COVID-19 patients: frequency and clinical presentation
Licensed Content Author	Noemi Güemes-Villahoz et al
Licensed Content Date	Aug 29, 2020
Type of Use	Thesis/Dissertation
Requestor type	non-commercial (non-profit)
Format	print and electronic
Portion	full article/chapter
Will you be translating?	no

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Mar 21, 2022

This Agreement between HOSPITAL CLINICO SAN CARLOS -- NOEMI GUEMES-VILLAHOZ ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	5273191066807
License date	Mar 20, 2022
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Graefe's Archive for Clinical and Experimental Ophthalmology
Licensed Content Title	Reduced macular vessel density in COVID-19 patients with and without associated thrombotic events using optical coherence tomography angiography
Licensed Content Author	Noemi Guemes-Villahoz et al
Licensed Content Date	May 7, 2021
Type of Use	Thesis/Dissertation
Requestor type	non-commercial (non-profit)
Format	print and electronic
Portion	full article/chapter
Will you be translating?	no

ELSEVIER

Nombre Noemi
Apellido Güemes-Villahoz
Instituto / Empresa UNIVERSIDAD COMPLUTENSE DE MADRID / Hospital Clinico San Carlos
Dirección Calle Profesor Martin Lagos SN
Código postal 28040
Ciudad MADRID
Estado / Territorio MADRID
País ESPAÑA
E-mail noemiguemes@gmail.com

Datos de la revista
Título de la revista
MEDICINA CLINICA
ISSN 2387-0206
Volumen156(11)
Número156(11)
Año2021
Páginas541-546
Título del artículo
Reduced retinal vessel density in COVID-19 patients and elevated D-dimer levels during the acute phase of the infection
Autor(es)
Guemes-Villahoz N, Burgos-Blasco B, Vidal-Villegas B, Donate-López J, Martín-Sánchez FJ, Porta-Etessam J, López-Guajardo L, Martín JLR, González-Armengol JJ, García-Feijoó J
Partes y uso del articulo
Partes del artículo Artículo completo
Cantidad de material (Si utiliza figuras, tablas, ilustraciones o vídeos) TODAS
Recuento de palabras (Si utiliza fragmentos)
¿Es autor del material? Sí
De no ser así, ¿participa el autor de Elsevier en su proyecto? Sí
Detalles sobre la participación del autor de Elsevier
Formato del material Impreso y Eléctronico
Traducira el material No
Idiomas a los que será traducido
Uso propuesto
Reutilización en una tesis doctoral / proyecto de final de grado