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Jordi Reina

Los linajes Victoria y Yamagata de los virus gripales B, desconocidos y poco valorados

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RESUMEN

El virus gripal B pertenece a la familia *Orthomyxoviridae* y al género *Influenzavirus* B. Presenta un genoma de tipo ARN negativo formado por unos 14.648 nucleótidos divididos en ocho segmentos distintos que codifican unas 11 proteínas. Antes de 1980 todos los virus de la gripe B pertenecían a un único linaje genético; pero en este año emergieron dos linajes antigénica y genéticamente distintos que se denominaron B/Victoria/2/1987 y B/Yamagata/16/1988. Se han podido demostrar procesos de intercambio genético intralinajes y entrelinajes; de ellos los mas frecuentes son aquellos en los que el linaje Victoria adquiere genes del linaje Yamagata. Se ha propuesto que las diferencias en las dinámicas evolutivas de los dos linajes se deban a las diferentes preferencias de unión de la hemaglutinina gripal al receptor celular. El linaje Victoria ha mostrado capacidad para unirse a los receptores celulares con restos de ácido siálico en las posiciones α-2,3 y α-2,6; mientras que el linaje Yamagata lo hace exclusivamente en las posiciones humanas α-2,6 del tracto respiratorio. La escasa circulación en los últimos meses podría haber contribuido a la eliminación ("extinción") temporal del linaje Yamagata. Desde 2017 la casi totalidad de las cepas de este linaje pertenecen al clado 3A, cuando con anterioridad se detectaban clados múltiples circulando. Aunque este clado 3A es diverso a nivel genético y ha adquirido mutaciones sustitutivas en el gen de la hemaglutinina, éstas no han determinado cambios antigénicos significativos que hayan obligado a sustituir su componente antigénico (B/Pukhet/3073/2013) en la vacuna gripal desde 2015.

Palabras clave: Gripe B; Linaje Victoria, Linaje Yamagata; Evolución genética

The Victoria and Yamagata Lineages of Influenza B Viruses, unknown and undervalued

ABSTRACT

The influenza virus B belongs to the family Orthomyxoviridae and to the genus *Influenzavirus* B. It has a negative RNA-type genome made up of about 14,648 nucleotides divided into eight different segments that encode about 11 proteins. Before 1980 all influenza B viruses belonged to a single genetic lineage; but in this year two antigenically and genetically distinct lineages emerged which were named B/Victoria/2/1987 and B/Yamagata/16/1988. Intralineage and interlineage genetic exchange processes have been demonstrated; The most frequent of them are those in which the Victoria lineage acquires genes from the Yamagata lineage. It has been proposed that the differences in the evolutionary dynamics of the two lineages are due to the different binding preferences of influenza hemagglutinin to the cellular receptor. The Victoria lineage has shown the ability to bind to cell receptors with sialic acid residues at the α-2,3 and α-2,6 positions; whereas the Yamagata lineage does so exclusively in the human α-2,6 positions of the respiratory tract. Low circulation in recent months may have contributed to the temporary elimination ("extinction") of the Yamagata lineage. Since 2017, almost all of the strains of this lineage belong to clade 3A, when previously multiple circulating clades were detected. Although this clade 3A is diverse at the genetic level and has acquired surrogate mutations in the hemagglutinin gene, these have not determined significant antigenic changes that have made it necessary to replace its antigenic component (B/Pukhet/3073/2013) in the influenza vaccine since 2015.

Keywords: Influenza B; Victoria Lineage, Yamagata Lineage; Genetic evolution

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INTRODUCCIÓN

En 1940 se produjo un brote de una enfermedad respiratoria aguda en la población infantil que clínicamente era semejante a la gripe. De estos pacientes se consiguió aislar un virus nuevo que era serológicamente distinto del ya conocido virus gripal A; el nuevo virus se denominó virus de la gripe B [1]. Las manifestaciones clínicas de la gripe B son generalmente indistinguible de las causadas por la gripe A. Globalmente la gripe B se presenta de forma bianual, casi siempre posterior a la onda epidémica de la gripe A, y representa alrededor del 25-30% de todos los casos de gripe comunicados a nivel global, aunque su porcentaje oscila según las epidemias y las zonas geográficas consideradas [2-5].

Una de las principales características diferenciales de los virus gripales B es que el ser humano es su único reservorio natural y por ello existe la posibilidad de que pueda ser eliminado mediante programas intensivos y efectivos de vacunación universal [2-5]. Sin embargo, en 1999 se detectó en Holanda un brote de gripe B que afectó a cetáceos marinos (focas), comprobándose que la cepa humana ya se había introducido en esta población animal en 1995 [6]. De este modo, aunque la circulación endémica de la gripe B está restringida a los humanos, se observa de forma esporádica paso de cepas a otras especies animales; sin embargo no existe ninguna evidencia de un reservorio animal sostenible para los virus gripales B [7]. Esta restricción de huésped podría atribuirse, al menos en parte, a las interacciones humanas específicas de las proteínas NS1 y M1 [8,9].

El virus gripal B pertenece a la familia *Orthomyxoviridae* y al género *Influenzavirus* B. Presenta un genoma de tipo ARN negativo formado por unos 14.648 nucleótidos divididos en ocho segmentos distintos que codifican unas 11 proteínas (no codifica las proteínas PB1-F2 y PA-X presentes en el virus gripal A), siendo la nucleoproteína B (NB) de su exclusividad [5,10] (Figura 1). La evolución genética de los virus gripales B es inferior a la observada en las cepas de la gripe A; así se ha calculado que la tasa de sustitución de nucleótidos en cada segmento genético por año es de $0.14x10^{-3}$-$3.32x10^{-5}$ en la gripe B frente a valores de $2.68x10^{-3}$-$12.5x10^{-3}$ en el subtipo (H3N2) de la gripe A [11-13].

Antes de 1980 todos los virus de la gripe B pertenecían a un único linaje genético; pero en este año emergieron dos linajes antigénica y genéticamente distintos que cocircularon por todo el mundo. Estos linajes se denominaron B/Victoria/2/1987 y B/Yamagata/16/1988 (Tabla 1) [14-16]. El linaje Yamagata divergió en el año 2.000 dando lugar a 2 clados antigénicamente diferentes (clados 2 y 3) que cocirculan en la actualidad, exhibiendo patrones de alternancia antigénica en cada estación epidémica. Por su parte el linaje Victoria también presentó un proceso de divergencia mas tardío, año 2011, dando lugar a dos clados monofiléticos (clados 1A y 1B) dominantes [17-19].

Se han podido demostrar procesos de intercambio genético (reasortamiento) intralinajes y entrelinajes; de ellos los mas frecuentes son aquellos en los que el linaje Victoria adquiere

	Segmento genómico	Proteínas
1	PB1	Proteína Básica 1
2	PB2	Proteína Básica 2
3	PA	Proteína Ácida
4	HA	Hemagluteína
5	NP	Nucleoproteína
6	NB	Neuraminidasa
7	M1/BM2	Proteína de Matriz
8	NS1/NEP	Proteína no estructural Proteína exportación neclear

Figura 1 | **Representación esquemática de los segmentos genómicos y sus correspondientes proteínas del virus gripal B.**

genes del linaje Yamagata [13]. De esta forma sólo los segmentos PB1, PB2 y hemaglutinina (HA) se siguen manteniendo separados entre los dos linajes. Por el contrario, los segmentos NS1/NS2 de las cepas actuales derivan todos del linaje Victoria y el resto de segmentos (PA, NP, NA/NB, M1/BM2) derivan del linaje Yamagata [13,20]. Todo ello ha determinado que las cepas del linaje Yamagata sean genéticamente mas estables y conservadas, muestren menores derivas antigénicas y una menor selección evolutiva, en comparación con las rápidamente cambiantes cepas del linaje Victoria [5,13].

Los análisis filodinámicos de la HA del linaje Victoria han mostrado una población sometida a una elevada presión selectiva que le permite escapar de la inmunidad humana a través de la adquisición de sustituciones beneficiosas en este gen. De este modo se constituyen de forma continua cepas nuevas con capacidad para infectar tanto a los vacunados como a los previamente infectados, aumentando el espectro infectivo de las mismas [13]. En el caso del linaje Yamagata, se ha observado una población viral con una dinámica evolutivo mucho menor, lo que se debería a una menor presión selectiva de escape y por ello una menor deriva antigénica [13,17,18].

En la gripe B cada cluster de cepas antigénicamente dominantes tiene tan solo una presencia transitoria, existiendo durante un corto período de tiempo antes que sea reemplazado por otro grupo nuevo. Este proceso, que podría ser equivalente a la deriva antigénica de la gripe A, se constituye como un proceso de selección dirigido, como se muestra en la elevada tasa de cambios en la HA (incluyendo mutaciones no sinónimas) y en la identificación directa de un importante número de posiciones que experimentan esta selección positiva. Como consecuencia de ello, la selección inmune en la HA y quizás en

Tabla 1 **Principales características diferenciales entre los dos linajes de los virus gripales B.**

Característica	Victoria	Yamagata
Distribución por edades	Jóvenes	Adultos
Diversidad genética	Importantes cambios estacionales	Escasos cambios
Contagiosidad (Re)	Alta (1.13-1.27)	Baja (1.08-1.14)
Selección positiva	Intensa	Escasa
Deriva antigénica	Importante	Ocasional
Reasortamiento	Alto inter-linajes y baja intra-linajes	Baja inter-linajes y alta intra-linajes
Receptor celular preferente	α-2,3 y α-2,6 ácido siálico	α-2,6 ácido siálico

(Re) número reproductivo efectivo. Modificado de Vijaykrishna et al. [13]

las proteínas NB y NS, desempeña un papel primordial en la determinación del patrón de cambios evolutivos en las cepas de la gripe B [12].

La importancia de esta selección inmune se refleja en la alternancia en la circulación de los dos linajes. De este modo el linaje Victoria fue el predominante en el período 1987-1989 y el Yamagata durante la mayoría de los 90s. Este patrón de alternancia es compatible con el modelo que predice que un linaje predomina en la población viral hasta que la inmunidad de grupo dirigida contra él, ya sea obtenida por infección o vacunación, sea mayoritaria en la población, a partir de ese momento el otro linaje empieza a incrementar su presencia y dominancia pasando a ser dominante en poco tiempo [12]. De este modo los patrones y procesos de cambio evolutivo en la gripe B no sólo vienen determinados por la naturaleza de su interacción con el sistema inmune humano, sino que además reflejan de que modo los dos linajes interactúan entre ellos y con la propia gripe A [13,14].

Se ha propuesto que las diferencias en las dinámicas evolutivas y epidemiológicas de los dos linajes se deban a las diferentes preferencias de unión de la HA gripal al receptor celular [13]. Así el linaje Victoria ha mostrado capacidad para unirse a los receptores celulares con restos de ácido siálico en las posiciones α-2,3 (aviar) y α-2,6 (humano); mientras que el linaje Yamagata lo hace exclusivamente en las posiciones humanas α-2,6 del tracto respiratorio [21,22]. Estudios experimentales realizados en niños menores de 7 años han mostrado que las células de su tracto respiratorio expresan preferentemente los residuos α-2,3 y a menor nivel los de tipo α-2,6 y a medida que se hacen adultos se produce el viraje y el predominio de los α-2,6 en la población adulta; estos datos explicarían, entre otras situaciones, el predominio del linaje Victoria en la infancia y el Yamagata en los adultos de mayor edad; es decir la diferente distribución por edad de los dos linajes de la gripe B [13,23].

Estudios previos realizados en los virus gripales A han mostrado la existencia de una impronta inmunológica frente al primer subtipo de HA, de modo que las respuestas posteriores dependerán de esta primera respuesta [24]. Vieira et al. [25] han comprobado que este mismo fenómeno se produce entre los dos linajes de la gripe B. Las HA y neuraminidasa (NB) de los virus gripales B evolucionan mucho mas lentamente entre los linajes que entre los diferentes subtipos de la gripe A. Además, los epítopos conservados en cada linaje pero variables entre ellos, podrían ser la base de la protección por impronta inmune frente al linaje Yamagata. Mientras que esta impronta frente al linaje Yamagata podrían afectar a la distribución etaria de los pacientes, la no inducción de esta impronta frente al linaje Victoria, podría explicar la casi constante presencia del mismo en cada temporada, aunque a niveles variables en ellas [13,25-26].

Se ha postulado que los anticuerpos inducidos por una primera infección por el linaje Yamagata podrían actuar sobre epítopos que están mas conservados que los epítopos neutralizados por los anticuerpos inducidos por la infección por el linaje Victoria. Por ello la protección inducida por el linaje Yamagata frente al linaje Victoria es débil y no duradera; el linaje Yamagata a menudo induce bajos o indetectables niveles de anticuerpos frente a la HA del linaje Victoria. Sin embargo, la primoinfección por el linaje Victoria induce anticuerpos capaces de inhibir intensamente la HA del linaje Yamagata, sugiriendo que el linaje Victoria podría proteger la infección posterior por el linaje Yamagata, independientemente de la edad del paciente [26,27]. Esta protección parece sólo obtenerse a través de los anticuerpos frente a la HA, ya que se observó que la adquisición del gen de la NB por parte del linaje Victoria en la temporada 2000-2001, no aportaba protección frente a la cepa reasortante formada; este hecho implica que la protección no se obtiene con los anticuerpos anti-NB [28,29].

La aparición de la pandemia causada por SARS-CoV-2 y la aplicación de diversas medidas sociales e higiénicas ha determinado la no circulación de la mayoría de los virus respiratorios convencionales. Entre ellos se ha detectado una casi desaparición de los virus gripales y del VRS; así a partir de abril de 2020 se produjo una reducción del 99% de las detecciones gripales. Esta disminución se acompañó de la correspondiente disminución de la diversidad genética de todos los virus gripales [30].

Esta escasa circulación podría haber contribuido a la elimi-

nación ("extinción") temporal del linaje Yamagata de la gripe B, ya que desde ese mes apenas se han comunicado detecciones de este linaje [30]. Aunque debe mencionarse que se ha observado que los virus gripales B entran periódicamente en un estado "durmiente" durante intervalos prolongados. Así durante los años 90 el linaje Victoria prácticamente dejó de circular, excepto por un brote detectado en África, pero fue dominante a nivel global desde principios del año 2000 [5,23].

La disminución en la prevalencia global del linaje Yamagata podría indicar una mayor vulnerabilidad y una perdida progresiva de la capacidad de adaptación al huésped humano. Este linaje presente un índice de reproductividad efectivo (Re) menor (Tabla 1) que el linaje Victoria, de modo que las epidemias causadas por el linaje Yamagata presentan una fase de crecimiento lento y una trasmisión de corta duración. Este aspecto le hace a este linaje mucho más vulnerable a los factores que dificultan su trasmisión, como las medidas adoptadas para contener la expansión del SARS-CoV-2 [13].

Los patrones de circulación global de los virus gripales se han asociado con los patrones etarios de la propia infección. Tal y como se ha mencionado el linaje Yamagata afecta preferentemente a la población adulta mayor de 25 años, que como consecuencia de la pandemia ha viajado poco en los últimos 18 meses, no permitiendo la libre circulación y la expansión del mismo. Pero no sólo el comportamiento humano podría ser el responsable de esta drástica reducción en la detección del linaje Yamagata, sino que el propio virus parece haberse estancado genéticamente. De este modo desde 2017 la casi totalidad de las cepas de este linaje pertenecen al clado 3A, cuando con anterioridad se detectaban clados múltiples circulando [17,19]. Aunque este clado 3A es diverso a nivel genético y ha adquirido mutaciones sustitutivas en el gen de la HA, éstas no han determinado cambios antigénicos significativos que hayan obligado a sustituir su componente antigénico (B/Pukhet/3073/2013) en la vacuna gripal desde 2015 [30].

La irrupción de la pandemia del SARS-CoV-2 coincidiendo con un período de baja incidencia y baja diversidad antigénica, junto a la efectividad vacunal de los últimos años, podría haber favorecido la desaparición o eliminación, quizás no extinción, del linaje Yamagata de la gripe B al menos la durante la pandemia. Con la disminución de la circulación del nuevo coronavirus y la reaparición de los otros virus respiratorios, podremos comprobar si esta desaparición es sólo temporal o definitiva en base a la vigilancia activa tanto virológica como epidemiológica.

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CONFLICTO DE INTERESES

El autor declara no tener ningún conflicto de intereses

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Nirmatrelvir más ritonavir (Paxlovid) una potente combinación inhibidora de la proteasa 3CLpro del SARS-CoV-2

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RESUMEN

Todos los coronavirus, incluido el SARS-CoV-2, codifican dos proteasas necesarias para el procesado de las poliproteínas pp1a y pp1ab. La proteasa principal 3CL (quimiotripsina-like) da lugar a la formación de las proteínas nsp11/16. La proteasa 3CL se ha constituido como una de las posibles dianas terapéuticas para el desarrollo de fármacos antivirales frente al SARS-CoV-2 debido a su secuencia y estructura altamente conservada entre todos los coronavirus. Durante la pandemia del SARS-CoV-1 se identificó un derivado hidroximetilcetona (PF-00835231) con una intensa actividad inhibidora frente a la proteasa 3CL. Las modificaciones químicas posteriores dieron lugar al derivado PF-07321332 (nirmatrelvir) que ha mostrado una elevada eficacia antiviral frente al SARS-CoV-2. Los datos de la compañía indican que es capaz de reducir un 89% el riesgo de hospitalización y fallecimiento de los pacientes infectados con apenas efectos adversos. Su eficacia mejora si se administra por vía oral en las primeras 24-48 horas y la duración del tratamiento se ha establecido entre 3-5 días. La forma comercial lleva asociada el antiviral ritonavir que ha mostrado enlentecer el metabolismo de nirmatrelvir, alargando su vida media. Este antiviral sería eficaz frente a las actuales y futuras variantes virales, ya que la 3CL no se modifica en ellas. La FDA aprobó este antiviral en noviembre de 2021 y la EMA está en fase de evaluación final.

Palabras clave: SARS-CoV-2; PF-07321332; Nirmatrelvir; PaxlovidTM; antiviral.

Nirmatrelvir plus ritonavir (Paxlovid) a potent SARS-CoV-2 3CLpro protease inhibitor combination

ABSTRACT

All coronavirus, including SARS-CoV-2, encode two proteases needed for the processing of PP1A and PP1AB polyproteins. The main protease 3CL (chemotripsine-like) gives rise to the formation of NSP11/16 proteins. The 3CL protease has been constituted as one of the possible therapeutic targets for the development of antiviral drugs against SARS-COV-2 due to its highly conserved sequence and structure among all coronaviruses. During the SARS-COV-1 pandemic, a hydroxymethyl ketone derivative (PF-00835231) was identified with an intense inhibitory activity against the 3CL protease. Subsequent chemical modifications gave rise to derivative PF-07321332 (nirmatrelvir) which has shown a high antiviral efficacy against SARS-COV-2. The company's data indicate that it is capable of reducing 89% the risk of hospitalization and death of patients infected with hardly adverse effects. Its effectiveness improves if it is administered orally in the first 24-48 hours and the duration of treatment has been established between 3-5 days. The commercial form has been associated with the antiviral ritonavir that has shown the metabolism of nirmatrelvir, lengthening its average life. This antiviral would be effective against current and future viral variants, since 3CL is not modified in them. The FDA approved this antiviral in November 2021 and EMA is in the final evaluation phase.

Keywords: SARS-CoV-2; PF-07321332; Nirmatrelvir; PaxlovidTM; antiviral.

INTRODUCCIÓN

Tras la emergencia y pandemia del SARS-CoV-2 se estableció la necesidad urgente de desarrollar fármacos antivirales específicos frente al mismo y plataformas vacunales de elevada

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eficacia. En estos momentos las vacunas han ganado la batalla frente a los antivirales, sin embargo éstos últimos serán una herramienta esencial en el control de la pandemia [1,2]. Los últimos datos comunicados por el directorio ClinicalTrials.gov muestran la existencia de 4.371 estudios basados en múltiples antivirales frente al SARS-CoV-2 [3]. A pesar de ello, sólo algunos ya han alcanzado la fase clínica final como molnupiravir (Lagevrio™), un análogo ribonucleósido de la citidina [4], autorizado por la Agencia Europea del Medicamente en noviembre de 2021 [5], y alguno de los derivados de la hidroximetilcetona, como nirmatrelvir (PF-07321332), que ha obtenido recientemente una autorización de emergencia por esta misma agencia (16 de diciembre de 2021) [6].

EL SARS-COV-2 Y LAS PROTEASAS

Todos los coronavirus, incluido el SARS-CoV-2, se caracterizan por presentar un genoma de ARN de una sola cadena (de unos 30.000 nucleótidos) de tipo ARN-mensajero (con un extremo 5´-Cap y un extremo 3´-poliA), es decir de tipo positivo. A diferencia de otros virus ARN se transcriben dando lugar a una poliproteína de una sola cadena (virus monocistrónico) que precisa ser hidrolizada por las proteasas virales, dando lugar a cada una de las diferentes proteínas que lo constituyen [7,8].

El SARS-CoV-2 produce a partir de zona replicativa dos poliproteínas solapadas derivadas de los ORF1a y ORF1b designadas como pp1a (nsp1-nsp11) y pp1ab (nsp1-16). Estas proteínas son inicialmente procesadas por dos proteasas de cisteína virales específicas designadas como proteasa papaína-like (PLpro) (nsp3) y proteasa quimotripsina-like (3CLpro o main proteasa, Mpro) (nsp5) dando lugar a unas 11-16 proteínas intermediarias y no estructurales, incluyendo a la ARN-polimerasa ARN-dirigida (nsp12) La PLpro da lugar a las proteínas nsp1-4 y la 3CLpro a las proteínas nsp5-11/16 (Figura 1) [9,10].

La proteasa PLpro, está formada por 1.945 aminoácidos, determina la deubiquitinación de las proteínas celulares como el factor regulador 3 del interferón (IRF3), así como también inactiva el factor nuclear NF-kB que aumenta la respuesta celular de las células B (inmunidad humoral) [10]. La proteasa 3CLpro es un homodímero de 303 aminoácidos que está formada por tres dominios: dominio I (aminoácidos 8-101), dominio II (102-184) y dominio III (201-303); además cada monómero contiene dos regiones catalíticas N-terminal y C-terminal [11,12]. Esta proteasa hidroliza las uniones glicina-serina dentro de la secuencia de reconocimiento leucina-glicina-serina-alanina-glicina de la proteína pp1ab [1,10-12]. La integridad y funcionalidad de estas proteasas, altamente conservadas, es imprescindible y esencial en el proceso replicativo de estos virus [11-14].

La proteasa principal 3CLpro se ha constituido como una de las posibles dianas terapéuticas para el desarrollo de fármacos antivirales frente al SARS-CoV-2 debido a su secuencia y estructura altamente conservada entre todos los coronavirus [14-16]. Sin embargo, los primeros estudios realizados utilizando los inhibidores de la proteasa de forma individual frente al VIH lopinavir/ritonavir no han mostrado apenas eficacia en los adultos frente al SARS-CoV-2 [16,17]. Del mismo modo, el compuesto AG7088 que es un potente inhibidor de las proteasas 3CLpro de los rinovirus y picornavirus tampoco ha mostrado eficacia frente a las proteasas del SARS-CoV-2 [18-22]. Una de las ventajas de estos antivirales es que no se han descrito pro-

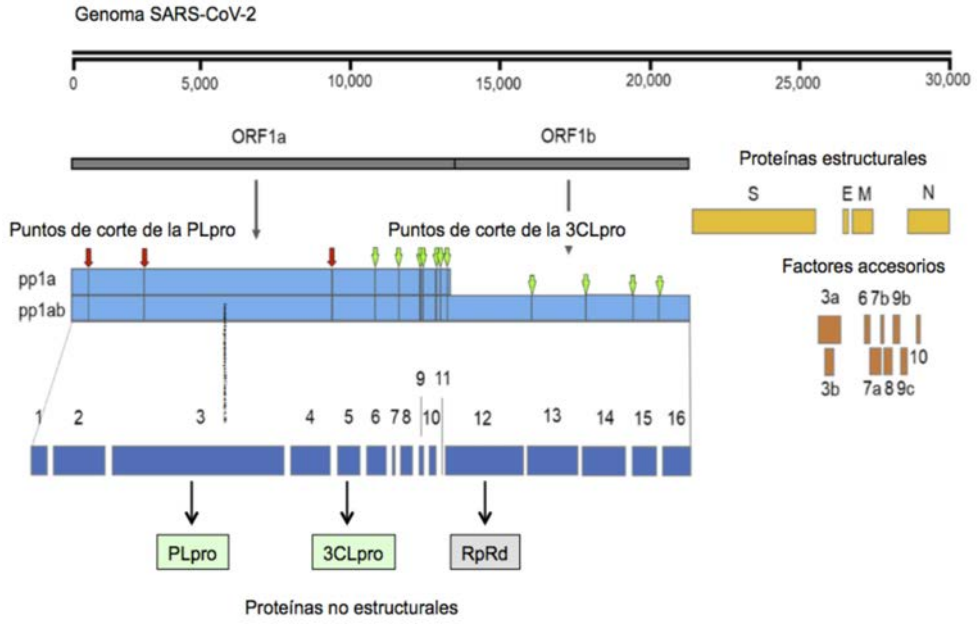

Figura 1 | **Estructura esquemática del genoma del SARS-CoV-2, proteasas y puntos de corte de las mismas (modificado de Zhu et al.[14]).**

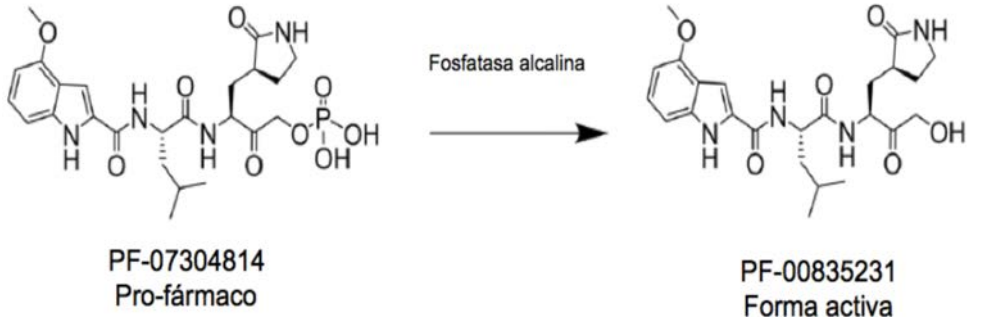

Figura 2 | **Activación por parte de las fosfatasas alcalinas celulares del pro-fármaco PF-07304814 a su forma activa PF-07321332 (nirmatrelvir).**

teasas humanas análogas a la 3CLpro, de modo que sus posibles inhibidores no afectarían a las células ni presentarían efectos adversos [19-23]. Además, estos fármacos serían eficaces tanto en las actuales como en las futuras variantes genéticas del SARS-CoV-2, dado que su diana molecular no está relacionada con la proteína S de este virus (proteína hipervariable) [1,2].

EL ANTIVIRAL NIRMATRELVIR

Durante la pandemia causada por el SARS-CoV-1 entre los años 2002 y 2003 Hoffman et al. [23] identificaron una pequeña molécula (derivado hidroximetilcetona o PF-00835231) con intensa actividad inhibidora de la proteasa 3CLpro. Dado que las secuencias de las proteasas 3CLpro del SARS-CoV-1 y SARS-CoV-2 presentan una identidad del 96% y del 100% en el sitio activo [10,15,24], se iniciaron los estudios sobre la utilidad de este compuesto para tratar a los pacientes con la COVID-19 [17].

Boras et al. [17] describieron el profármaco fosfatado de este compuesto (designado PF-07304814) que ha mostrado mayor absorción y biodisponibilidad (Figura 2). Así mismo estudiaron la eficacia de este fármaco y observaron una intensa actividad antiviral frente al SARS-CoV-2 tanto en cultivos celulares como en ratones a una concentración efectiva de 0.5 µM. Este dato permite predecir que se alcanzaría esta concentración terapéutica en el ser humano con una dosis de 500 mg en infusión continua durante 24 horas. Así mismo han observado, en cultivos celulares, el efecto sinérgico entre el compuesto PF-00835231 y remdesivir, un análogo de la adenosina que interrumpe la replicación viral [19]. De este modo se podría utilizar esta combinación sinérgica en los pacientes graves o con elevadas cargas virales [17].

Hammond et al. [25] ha presentado recientemente los datos preliminares de un estudio con el fármaco antiviral nirmatrelvir, que es un potente inhibidor de la proteasa 3CLpro del SARS-CoV-2. Este fármaco es una modificación química del original descrito por Hoffman et al. [23], que se administra a una dosis experimental de 300 mg por vía oral cada 12 horas durante un período de 3 o 5 días. En el estudio aleatorizado, doble ciego, en Fase 2/3 EPIC-HR (Evaluation of Protease Inhibition for COVID-19 in High Risk Patients) realizado en 1.219 pacientes adultos no hospitalizados con infección demostrada por SARS-CoV-2 y elevado riesgo de progresión a enfermedad grave, por patologías previas, se demostró una reducción del 89% en el riesgo de hospitalización o fallecimiento por cualquier causa frente a placebo en pacientes tratados en los 3 primeros días desde el inicio de los síntomas. De este modo sólo el 0,8% de los pacientes que recibieron nirmatrelvir fueron hospitalizados durante los 28 días siguientes al inicio del tratamiento (sin ningún fallecimiento) (3/389 pacientes), mientras que en el grupo del placebo se hospitalizaron el 7% (con siete fallecimientos entre ellos), siendo estos datos estadísticamente significativos (27/385 pacientes) ($p<0.0001$).

Datos similares se observaron en los pacientes tratados en los primeros 5 días desde inicio de los síntomas. Sólo se hospitalizaron el 1% de los tratados con nirmatrelvir durante los 28 días siguientes, mientras que si lo hicieron el 6,7% del grupo placebo ($p<0,0001$). Globalmente a los 28 días del inicio de los síntomas no se comunicó ningún fallecimiento entre los que recibieron el inhibidor 3CLpro nirmatrelvir, pero si ocurrió en el 1.6% (10 casos) de los que recibieron placebo. Los efectos adversos detectados fueron muy parecidos entre los dos grupos (19-21%), la mayoría de ellos considerados como moderados. Los efectos adversos graves se produjeron en el 1,7% de los tratados con nirmatrelvir frente al 6.6% del grupo placebo y el cese de tratamiento se detectó en el 2.1% de los tratados con el antiviral (4,1% grupo placebo) [25].

Por su parte Owen et al. [26] han observado en ratones adaptados al SARS-CoV-2 que el tratamiento a la dosis de 300 mg/kg/2 veces al día les protegía de la infección intranasal por este virus. Además, comprobaron una disminución significativa de la carga viral en el tracto respiratorio y parénquima pulmonar, confirmando su potente efecto antiviral.

En un ensayo clínico de seguridad, tolerabilidad y farmacocinética se observó en adultos sanos (ClinicalTrial.gov. NCT04756531) que dosis bajas de ritonavir (100 mg) enlentecían el metabolismo y la desactivación de nirmatrelvir por la citocromo-oxidasa hepática, de modo que permanecía activo

durante períodos mas prolongados y en concentraciones mas elevadas [25,26]. Además, nirmatrelvir se ha mostrado altamente eficaz en el bloqueo de la transcripción de las principales variantes del SARS-CoV-2 descritas hasta la actualidad [22]. La FDA aprobó la combinación nirmatrelvir más ritonavir (Paxlovid™) el 6 de noviembre de 2021 para su comercialización y administración en humanos. La dosis recomendada sería de 300 mg del nuevo antiviral (dos pastillas de 150 mg) junto a una pastilla de ritonavir (100 mg) dos veces al día durante 5 días [27].

La administración oral de este antiviral y sus escasos efectos adversos podrían permitir su utilización generalizada al inicio de los primeros síntomas de infección por SARS-CoV-2. Como la mayoría de otros antivirales frente a los virus respiratorios, el tratamiento debería iniciarse en las primeras 24-48 horas tras la aparición de los síntomas, coincidiendo con la fase replicativa mas intensa del virus. Su utilización podría evitar las formas graves de la COVID-19 y disminuir los ingresos hospitalarios, tanto en pacientes vacunados como en los no vacunados e incluso en las reinfecciones por variantes distintas a la primoinfección, como la ómicron, actualmente en circulación masiva [28,29].

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CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses

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Review

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Effectiveness of cell culture-based influenza vaccines compared with egg-based vaccines: What does the literature say?

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ABSTRACT

Introduction. Influenza vaccination is an effective way of reducing the burden of seasonal influenza. Chicken egg embryos are the most common source of influenza vaccines, but cell culture production has emerged as an alternative that could be advantageous. This article reviews the available literature on the efficacy/effectiveness of cell culture-based influenza vaccines compared with egg-based vaccines.

Methods. We conducted a review of the actual literature and analyzed those studies comparing the effectiveness of cell culture-based and egg-based vaccines in the last ten years.

Results. Eight studies were analyzed; 1 was a clinical trial and 7 were retrospective cohort studies. The clinical trial found no significant differences in the efficacy of both vaccines with respect to placebo. The results of the observational studies were inconsistent and relative effectiveness varied among studies, even though most were performed during the same season, and in some cases, in the same region and using the same data records. Furthermore, in most studies, the comparisons between vaccines were not statistically significant.

Conclusions. There is insufficient evidence that cell culture-based vaccines are superior to egg-based vaccines in terms of efficacy/effectiveness.

Keywords: Influenza, influenza virus, egg-based vaccine, cell culture-based vaccine.

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Efectividad de la vacuna antigripal generada en cultivo celular frente a la producida en huevo: ¿Qué dice la literatura actual?

ABSTRACT

Introducción. La vacunación frente a la gripe es el método más efectivo para reducir el impacto de la gripe estacional. Los embriones de huevo de gallina son el método más común de fabricación de vacunas antigripales, pero la propagación en cultivos celulares ha emergido como una alternativa que podría ofrecer alguna ventaja. El objetivo de este artículo es hacer una revisión de la literatura disponible sobre la efectividad de vacuna antigripal generada en cultivos celulares frente a la vacuna producida en huevo.

Métodos. Se realizó una búsqueda bibliográfica de los estudios comparativos entre la vacuna propagada en cultivos celulares y la producida en huevo con respecto a su efectividad publicados en los últimos diez años.

Resultados. De los siete estudios analizados, uno fue un ensayo clínico y seis fueron estudios de cohortes retrospectivos. Los resultados del ensayo clínico mostraron que no existían diferencias significativas en cuanto a la eficacia de ambas vacunas. Con respecto a los estudios observacionales, los resultados fueron poco consistentes, con efectividades relativas que fueron muy diferentes entre estudios a pesar de que la mayoría se realizaron durante la misma temporada, y en algunos estudios, en la misma región y utilizando el mismo registro de datos. Además, en la mayoría de los estudios no hubo significación estadística.

Conclusiones. No existen evidencias suficientes de que la vacuna producida en cultivo celular sea superior a la generada en huevo con respecto a su efectividad.

Palabras clave: Influenza, virus gripe, cultivos celulares, huevo, vacunas

INTRODUCTION

The influenza virus is one of the most serious human pathogens and one of the leading causes of acute respiratory infections. Seasonal influenza causes between 4 and 50 million symptomatic cases in the European Union each year, affecting about 10%-30% of the population and causing the deaths of between 15,000 and 70,000 people annually [1].

Vaccination is an effective method of preventing influenza infections and associated complications [2]. Influenza vaccines consist of 3 (trivalent) or 4 (quadrivalent) strains of influenza virus (A/H1N1, A/H3N2, B/Yamagata, B/Victoria). In February of each year, the World Health Organization (WHO) determines the annual composition of influenza vaccines for the following season in the northern hemisphere based on global epidemiological data of virus strains circulating in the previous period. The viral formulation is then distributed to vaccine manufacturers who produce the viruses, generating enough doses for the population.

Currently, influenza vaccines are mainly produced in fertilized chicken eggs, and the efficacy and safety of this system is well established. However, egg-based manufacturing also has some disadvantages, such as limited production capacity, prolonged production time, and the possibility of allergic reactions caused by egg-derived proteins. The isolation and propagation of the virus in eggs can also cause mutations in the amino acids around the binding site of the hemagglutinin (HA) receptor that could affect their effectiveness [3,4].

Novel alternatives to the manufacture of the influenza vaccine in eggs have been in development for some time [5]. The 2 formulas most commonly used in production worldwide are the generation of recombinant vaccines and propagation in cell lines [6-8]. Since the end of 2019, a cell-based influenza vaccine has been marketed in Spain [9]. This is a quadrivalent vaccine produced in mammalian cells (Madin-Darby Canine Kidney, MDCK) that have been shown to be suitable for the propagation of the virus [10]. This methodology has a number of advantages in the propagation process. For a start, it does not appear to make significant changes in the HA amino acid sequence [11]. Moreover, cell lines are widely characterized and can be stored for future use without repeating a full-range test. Because some viruses grow better in cells, the time required for generating large-scale rearrangements is reduced and scalability is improved. However, this system also has some limitations, cells must be free of adventitious viruses, which requires extensive screening of cell lines [12]. Furthermore, the implementation of this system would involve the creation of new production facilities [12].

In recent years, various studies have analyzed how cell culture-based influenza vaccines function, particularly in terms of tolerability and immunogenicity [6,13-15]. However, to date no clinical trials have directly compared the efficacy/effectiveness of cell-propagated influenza vaccines with vaccines generated in chicken eggs. A 3-arm randomized clinical trial that studied vaccines produced in embryonic hen eggs, cell culture-generated vaccines, and placebo has been published, but the vaccines were not compared directly against placebo. The data available to date are based mainly on observational single-season studies which are difficult to compare due to methodological differences (case definition, virological confirmation) and the high variability of the influenza virus in each season.

In an attempt to determine the comparative efficacy/effectiveness of cell culture-based and egg-based influenza vaccines, our group of experts in epidemiology, pediatrics, family medicine, and scientific methodology analyzed the evidence published in the last 10 years. This article presents our results and conclusions.

METHODS

We conducted a literature search of the available scientific studies comparing the efficacy/effectiveness of cell culture-based and egg-based influenza vaccines in MEDLINE/PubMed and Scopus between November 2010 and November 2020, restricted to articles published in English or Spanish. In total, 140 articles were retrieved. After excluding publications with the format of reviews, comments on articles, editorials and opinion articles, 8 publications were finally included in the analysis: 1 clinical trial and 7 observational studies. The authors met via teleconference and discussed about the selected bibliography.

RESULTS

Analysis of studies comparing egg-based influenza vaccines and cell culture-based vaccines. Table 1 summarizes the characteristics of the 7 observational studies in which the effectiveness of cell-based influenza vaccine was compared with chicken egg-based influenza vaccine. The efficacy of both vaccines with respect to placebo was analyzed in only 1 study [16]. This study, published in 2010, also assessed the tolerability and immunogenicity of the vaccines using a randomized, observer-blind, placebo-controlled design in individuals aged 18 to 49 years. Adults from the US, Finland, and Poland were included during the 2007-2008 season. Participants were randomized to receive (1: 1: 1) trivalent cell-cultured vaccine, trivalent egg-based vaccine, and placebo. Influenza was defined as influenza-like illness (ILI) with a temperature of ≥ 37.8ºC plus sore throat/cough and confirmation by PCR. The results of the study that included 11,404 randomized subjects (3,776 received cell culture-derived vaccine, 3,638 egg-derived vaccine, and 3,843 placebo) showed that both vaccines were effective and differences were not statistically significant. Overall efficacy for all strains compared to placebo was 69.5% for cell culture-derived vaccine and 63.0% for egg-based vaccine [16]. The two vaccines also showed high immunogenicity and were well tolerated [16]. It is important to note that, since this was not a head-to-head clinical trial of the two types of vaccines but instead a comparison against placebo, the results lacked sufficient statistical power to make a valid direct com-

parison the trial. Another limitation is that this was an observer-blind trial which may have led to multiple biases. Nor were details provided on whether the cell culture-based influenza vaccine had previously been adapted in egg, a process that was common in the early years of cell-based vaccine production [4]. The efficacy of the vaccines was analyzed during 1 season only, despite the well-known ability of the influenza virus to undergo significant antigenic variations every year [17].

In the last 10 years, 6 observational studies have evaluated the effectiveness of the influenza vaccine produced in fertilized hen eggs compared to cell culture-derived vaccines. Most were conducted in the 2017-2018 season, which saw a high incidence of influenza cases. DeMarcus et al. [18] performed a study using a negative test design that included PCR-confirmed ILI cases that were treated at U.S. Department of Defense medical centers between October 1, 2017, and April 28, 2018. The study included 4,037 samples from 1,757 cases (43.5 %) and 2,280 controls. Thirty percent (531) of the cases had been vaccinated, 11% with cell culture-derived vaccines and 19% with egg-derived vaccines. Subjects of all ages (from 6 months to over 65 years), with a mean age of 24 years, were included. Statistically significant differences in favor of the egg-based vaccine were only found for H1N1 strains, which showed efficacy rates of 86% versus 61% for all age groups and 88% versus 56% in children (Table 1). The analysis of all strains in all age groups showed a higher, but not statistically significant, relative vaccine effectiveness (VE) for the egg-derived vaccine (VE 53 % vs. 46%) [18]. An evaluation of the methodological aspects of the article reveals a significant difference in the age distribution of patients who received each type of vaccine: most of the children who were vaccinated received the egg-derived vaccine (79.0%)[18]. Thus, since the confidence intervals will be smaller in this group, it is easier to achieve statistically significant effectiveness. Furthermore, comorbidities were not taken into account during the study. Another possible bias is that the study was conducted in members of the U.S. Department of Defense, a population that generally has better health than the real-world population in whom the flu vaccine is usually targeted. The timing of vaccination may also have had an effect, since the chicken egg-derived vaccine was administered before the cell culture-derived vaccine. This may be of great importance, since the effectiveness of the influenza vaccine is estimated to fall by 6%-12% each month [19], thus favoring greater VE in the cell culture-based vaccine. The study has other limitations, such as a small sample size, a restrictive subanalysis, and follow-up for a single season.

Another study conducted in the U.S. in the 2017-2018 season included hospitalized subjects aged ≥ 4 years enrolled in the California Kaiser Permanente registry between October 1, 2017 and May 31, 2018 [20]. The effectiveness of the influenza vaccine against hospitalization for laboratory-confirmed influenza was assessed in 5,239 individuals who received the egg-based vaccine, 232 people who received the cell culture-based vaccine, and 2,661 people who were not vaccinated. No differentiation was made between trivalent or quadrivalent vaccines. After analyzing the results, a statistically significantly greater relative VE was observed with the egg-based vaccine in patients < 65 years of age for the A/H3N2 strain only, with negative effectiveness (-44%; 95% CI : -99, -4) [20]. However, since it was an inactivated vaccine and cannot cause the disease, this outcome has no clinical implications. The study has little statistical power due to its small sample size. Furthermore, it has multiple biases, such as the exclusion from the analysis of patients without confirmed influenza and the fact that vaccinated patients had more comorbidities. The interval between the onset of symptoms and the influenza test was not reported. Moreover, a remarkably smaller number of patients received the cell culture-based vaccine, revealing an uneven distribution of vaccines. Overall, the results of this article are inconsistent.

Klein *et al.* [21] also used the Californian Kaiser Permanente patient registry during the 2017-2018 season. In this study, the relative VE of the quadrivalent cell-based influenza vaccine was compared to the egg-based vaccine in standard, trivalent, and quadrivalent doses, in patients aged 4 to 64 years. Although the study did not show statistically significant differences with respect to relative VE, the egg-based vaccine showed superiority in patients younger than 65 years (Table 1). Again, however, the study found a significant imbalance in the distribution of vaccines in the children's group. While 50% of individuals who received the cell culture-based vaccine were children between the ages of 4 and 17, only 23% of the individuals who received the egg-based vaccine belonged to this age group.

The study conducted by Boikos *et al.* [22] estimated the relative VE of the cell-cultured quadrivalent vaccine compared to the egg-derived vaccine in the US during the 2017-2018 season, using electronic medical records from primary care practices with a catchment population of over 55 million people. All patients ≥ 4 years who presented with ILI without microbiological confirmation of influenza, who were seen at any of the centers included in the database, and who had been vaccinated at least 14 days before the onset of symptoms were included in the study. A multivariate logistic regression was performed adjusting for possible confounding factors defined *a priori*. Sensitivity analysis was also performed using propensity score matching and analysis by age subgroups. A total of 1,353,862 subjects were included, 93% of whom had received the egg-based vaccine and 7% the cell culture-based vaccine [22]. The cell culture-based vaccine showed a significantly higher relative VE of 36.2% (95% CI : 26.1, 44.9), consistent with the primary analysis. It is essential to stress that the definition of ILI is decisive in these studies, as it may influence the results. Although the study found a concordance between ILI cases included in the study and influenza confirmed by the Centers for Disease Control and Prevention (CDC) laboratory, direct extrapolation is complicated because multiple circulating influenza-like illnesses follow the same seasonality as influenza, although influenza vaccines are only effective against influenza. It should also be noted that the allocation of vaccines should be random for both vaccines, so the penalization for not including PCR confirmation of influenza should be the

Table 1 **Characteristics of the studies comparing egg-based influenza vaccines and cell culture-based vaccines**

Type of study	First author, year of publication [reference]	Number of patients	Primary variable	Age group	Patients included	Effectiveness (percent) (95% CI)
Clinical trial	Frey S, 2010 [16]	11,404	ILI and PCR influenza confirmation	Patients 18-49	United States, Finland, and Poland	rVE versus placebo Cell culture-based trivalent: - 83.8 (61.0, 97.5) against vaccine strains - 69.5 (55.0, 97.5) against circulating strains Egg-based trivalent: - 78.4 (52.1, 97.5) against vaccine strains - 63 (46.7, 97.5) against circulating strains
Retrospective observational study	Klein NP, 2020 [21]	3,053,248	Influenza confirmed by PCR	Patients aged 4 to 64 years	Kaiser Permanente Members (Northern California, USA)	rVE against A virus (cell culture vs egg) 4-64 years: 8.0 (-10, 23) 4-17 years: 17.8 (-6.2, 36.4) 18-64 years: -5.8 (-36.1, -17.7) rVE against B virus (cell culture vs egg) 4-64 years: 39.6 (27.9, 49.3)[a] 4-17 years: 42.3 (28.4, 53.5)[a] 18-64 years: 21.4 (-36.1, 17.7)
	Boikos C, 2020 [22]	1,353,862	Visit to primary care for ILI	Patients older than 4 years	US Primary Care electronic medical records (EMR)	rVE (cell culture vs egg) 18- 64 years: 26.8 (14.1, 37.6) ≥ 65 years: -7.3 (-51.6, 24.0) 4-17 years: 18.8 (-53.9, 57.2)
	DeMarcus L, 2019 [18]	4,037	Influenza confirmed by PCR and/or culture	≥ 6 months	Outpatient records Department of Defense	rVE versus non-vaccinated All ages: - Non-significant differences for H3N2 - H1N1 86 (68, 91) for egg-based 61 (38, 76) for cell culture-based Children (6 months-17 years) - Non-significant differences for H3N2 - H1N1 88 (80, 93) for egg-based 56 (15- 77) for cell culture-based
	Bruxvoort KJ, 2019 [20]	8,132	Hospitalization for influenza confirmed by PCR	Patients > 4 years to < 65 Patients > 65 years	Kaiser Permanente Members (California, US)	rVE (cell culture vs egg) < 65 years: 43 (-45, 77) > 65 years: 61 (-63, 91)
	Izurieta HS, 2019 [23]	> 16,000,000	Primary: Hospital records (admissions + emergency)	Patients ≥ 65 years	Medicare members (US)	rVE (cell culture vs egg) vs hospitalizations All ages: - 11 (7.9, 14.0)
	Izurieta HS, 2020 [24]	12,777,214	Primary: Hospital records (admissions + emergency)	Patients ≥ 65 years	Medicare members (US)	rVE (cell culture vs egg) vs hospitalizations All ages vs hospital events: - 0.8 (-4.6, 5.9.) All ages vs hospitalizations - 3.4 (-3.6, 9.8)
	Izurieta HS, 2020, [25]	12,700,000	Primary: Hospital records (admissions + emergency)	Patients ≥ 65 years	Medicare members (US)	rVE (cell culture vs egg) vs hospitalizations All ages vs hospital events: - 2.8 (-2.8, 8.2)

[a] $p < 0.0001$; rVE: relative vaccine effectiveness; CI: confidence interval

same. Furthermore, the statistical analysis has some shortcomings. The distribution of patients receiving each vaccine is uneven, with older white people more frequently receiving the cell culture-based vaccine. Models for selecting variables based on statistical ratios were used when it would have been more appropriate to use a multivariate analysis. In conclusion, many factors of this study were not properly controlled.

Another comparative study of the 2 vaccines during the 2017-2018 season in the United States was performed by a US team led by Héctor Izurieta [23]. This was a retrospective study that included Medicare beneficiaries ≥ 65 years of age who had received an influenza vaccine (egg-based/cell culture-based quadrivalent, egg-based high-dose, adjuvanted, or standard-dose trivalent). The investigators analyzed the relative VEs of each type of vaccine in the prevention of influenza-related hospital events (without diagnostic conformation) including hospitalizations and emergency visits. The results showed that the quadrivalent cell culture-based vaccine was more effective than the standard-dose quadrivalent egg vaccine (relative VE 10%, 95% CI: 7, 13). However, there were no statistically significant differences with the high-dose trivalent vaccine, also produced in eggs (relative VE 11 % vs. 9%, respectively) [23]. It is important to note that influenza cases were not confirmed by PCR in this study. Moreover, the predominant A strain in the 2017-2018 season was H3N2, and there was no concordance with the B strain included in the trivalent vaccine. Another factor to consider with regard to the study methodology is the population included. Elderly people (≥ 65 years) are candidates for the high-dose vaccine as a first option. However, since the study took place in the US, clinicians selected the vaccine and the antigenic load to administer to patients. Although the analysis is correct from a statistical point of view, with an adjustment for previously measured confounders, the design does not allow adjustment for unmeasured confounders.

During the following season, 2018-2019, the team led by Izurieta *et al.* also conducted comparative research among vaccines following the same methodology as in their previous study [24]. The study, that included nearly 13 million Medicare beneficiaries over the age of 65, showed that egg-produced quadrivalent vaccines were more effective than cell culture-generated vaccines (relative VE 2.5 %; 95 % CI: -2.4, 7.3) although the results were not statistically significant [24].

The team led by Héctor Izurieta has recently published a new study comparing the effectiveness of influenza vaccines in the 2019-2020 season [25]. This study followed the same design as described in previous seasons [23,24], but added a quadrivalent recombinant HA-based influenza virus vaccine, delivering a high HA dose (45 μg per strain) [26]. In that season, the quadrivalent vaccine produced in mammalian cells was not significantly more effective than the egg-based vaccine (2.8 %, 95 % CI: -2.8, 8.2).

DISCUSSION

In the last two decades, the use of cell culture platforms for the production of influenza vaccine has been explored as an alternative to the generation of vaccines using fertilized chicken eggs. However, until now, the clinical effectiveness of these two vaccines has not been directly compared in clinical trials. In this critical review of the literature, we have analyzed studies published over the past 10 years comparing the effectiveness of the two vaccines, including 7 observational studies and a clinical trial in which both vaccines were compared with placebo. We conclude that there is insufficient evidence to show that the vaccine produced in cell culture is superior to that generated in egg.

The clinical trial developed by Frey et al. [16] was designed to demonstrate the efficacy of cell culture-based and egg-based vaccines separately against placebo. This approach was adopted to decrease the sample size required for the placebo group. However, this clinical trial was not designed to allow a direct comparison between the 2 types of vaccines. The European Medicines Agency (EMA) also recommends in its guidelines for the development of influenza vaccines that trials should be double-blind and include other secondary variables, such as hospitalization, all-cause mortality, all-cause pneumonia, and otitis (in children), factors not addressed in this study [27]. In brief, the results of this trial do not allow conclusions to be drawn on the efficacy of cell culture-based influenza vaccine compared with egg-based vaccine.

The results of the 7 observational studies included in our review, mostly conducted in the 2017-2018 influenza season and all in the U.S., are inconsistent. The relative VEs varied widely among these studies despite the fact that some were performed in the same population, and in some cases, using the same data records (Table 1). While some studies favored the cell culture-based vaccine, in others, the relative VE was better for egg-based vaccine. It should also be noted that many of the studies do not use ILI together with laboratory confirmation of influenza by PCR as their primary variable, as recommended by EMA for the development of influenza vaccines [27].

When the 3 studies carried out by the team of Izurieta *et al.* during 3 consecutive seasons (2017-2020), in which the same population was included and the same methodology followed, are compared, the results again lack consistency [23-25]: the effectiveness data for the 2017-2018 season favored cell culture-based vaccine, while in the following two seasons, a better trend was observed for egg-generated vaccines. In most cases the differences were not statistically significant, so no clinical conclusions can be drawn.

Differences on effectiveness of influenza egg-based vaccines versus cell culture-produced vaccines have been analyzed on previous reviews [28]. This study evaluated recent data regarding the effectiveness of both vaccines and concluded that the global results seem to support greater effectiveness, backed by greater antigenic stability of cell culture-derived vaccines over egg-based vaccines [28]. However, it is important to point out that the analysis that led to these conclusions were not mainly based on studies directly comparing both kinds of vaccines, but on individual effectiveness studies

that are difficult to compare due to the differences in their methodological designs.

Recently, 3 recognized scientific bodies have conducted a comprehensive review of the available evidence for new influenza vaccines. The European Center for Disease Prevention and Control (ECDC) has conducted a comprehensive evaluation of the quality of evidence using GRADE (Grading of Recommendations Assessment, Development, and Evaluation) methodology [29]. The following vaccines were analyzed in a systematic literature review: MF59®-adjuvanted vaccine, cell culture-based vaccine, high-dose vaccine, and recombinant vaccine. The ECDC concluded that cell culture-based vaccines are effective compared to non-vaccination. However, no superiority conclusions were established regarding the effectiveness of these vaccines compared to egg-based vaccines, as the available data are limited. The German Standing Committee on Immunization (STIKO) has also analyzed the current literature on influenza vaccines using GRADE methodology. Their paper concludes that the cell culture-based vaccine is likely to be effective when compared with placebo and that data comparing its effectiveness with that of egg-based vaccines are limited. The National Immunization Advisory Committee of Canada (NACI) has also issued a supplementary statement to the Canadian immunization guideline providing guidance on the use of standard-dose quadrivalent vaccine produced in cell cultures [30,31]. In this case, recommendations were based on a systematic review conducted according to the PRISMA criteria, following standard NACI methodology for grading evidence [32]. It concluded that there were no consistent or statistically significant results to confirm that the cell culture-propagated vaccine is more effective than egg-produced vaccine.

Based on all studies evaluated, it is impossible to establish differences in vaccine effectiveness between egg-based and cell culture-based vaccines, given the lack of significant differences. Furthermore, the results of multiple studies diverge. While some show the superiority of cell culture-propagated vaccines, others have found that egg-based vaccine is more effective. Methodological differences are among the factors that account for the inconsistent results. It seems clear that coherent study designs are desirable. Sample size should be larger to help achieve statistical significance between the different groups. Another variable to consider is the correct definition of ILI and the confirmation of influenza by PCR. Since the diagnosis of ILI is very heterogeneous and varies from specialist to specialist, failure to obtain PCR confirmation can generate significant biases. It should also be remembered that influenza vaccines are only effective against influenza disease.

Finally, another obvious factor is that the influenza virus is very complex and highly susceptible to variations. All of the studies evaluated a single season; however, the characteristics of the influenza virus and its infectious behavior must be followed up over multiple seasons in order to determine the real effectiveness of influenza vaccines.

Even though every effort was made to deliver a comprehensive review of the available literature using an exhaustive and significative literature search strategy, this review has limitations. We only report results from articles published in English or Spanish from the past 10 years. In addition, our results are limited by the quality of the identified studies, but it should be remembered that the objective of this review was in fact to analyze the published evidence. On the other hand, as noted previously, the results are bound by the inherent limitations of the studies included in the literature review such as outcomes, case definitions, mode of data collection and detection methods for laboratory-confirmed influenza. A timely meta-analysis/systematic review of this topic would provide stronger evidence and would help resolve possible divergences in the literature.

CONCLUSION

Based on the studies evaluated, no differences can be established in the effectiveness of the cell culture-based and the egg-based influenza vaccines. Independent analyses conducted by NACI, ECDC and STIKO also reached the same conclusion. Future studies should take into account the high complexity of the influenza virus and be designed by consensus, with a larger sample size, precise definition of ILI, including PCR confirmation of influenza, and, above all, follow-up over multiple seasons to determine superiority in vaccine effectiveness.

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CONFLICT OF INTEREST

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Efficacy of early use of remdesivir: a systematic review of subgroup analysis

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ABSTRACT

Introduction. A possible benefit has been suggested for early treatment of severe coronavirus disease 2019 (COVID-19) with remdesivir. The efficacy of this drug is controversial and could significantly influence the efficiency in healthcare systems. The objective is the methodological interpretation of subgroup analyzes according to starting of remdesivir treatment with respect to symptom onset of COVID-19.

Methods. A search in Pubmed® database was performed. Randomized clinical trials (RCTs) with subgroup analysis regarding early and late use of remdesivir were selected. All endpoints were assessed using two methodologies. First methodology considered statistical interaction, pre-specification, biological plausibility, and consistency of results. Second methodology was a validated tool with preliminary questions to discard subset analysis without relevant minimum conditions, and a checklist with recommendations for applicability.

Results. A total of 54 results were found and five RCTs were selected. According first methodology, consistent heterogeneity was only found in time to clinical improvement and better clinical status score at day 15 for patients with severe COVID-19 and <7 days of symptoms. About second methodology, these results about early use of remdesivir may be applied to clinical practice with caution.

Conclusions. We developed a systematic search and application of an established methodology for interpretation of subgroup analysis about early use of remdesivir. Results in severe COVID-19 suggested that early use of remdesivir provides a greater benefit in <7 days of symptoms for time to clinical improvement and better clinical status score at day 15. Future studies could use 7-day cut-off of symptoms to evaluate remdesivir.

Keywords: COVID-19; remdesivir; clinical decision-making; subgroup analysis; drug evaluation.

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Eficacia del uso temprano de remdesivir: una revisión sistemática de análisis de subgrupos

RESUMEN

Introduction. Se ha sugerido un posible beneficio para el tratamiento temprano de la enfermedad grave por coronavirus 2019 (COVID-19) con remdesivir. La eficacia de este fármaco es controvertida y podría influir significativamente en la eficiencia de los sistemas sanitarios. El objetivo es la interpretación metodológica de los análisis de subgrupos según el inicio del tratamiento con remdesivir respecto al inicio de los síntomas de la COVID-19.

Material y métodos. Se realizó una búsqueda en la base de datos Pubmed®. Se seleccionaron ensayos clínicos aleatorizados (ECA) con análisis de subgrupos respecto al uso temprano y tardío de remdesivir. Todas las variables se evaluaron mediante dos metodologías. La primera metodología consideró la interacción estadística, pre-especificación, la plausibilidad biológica y la consistencia de los resultados. La segunda metodología fue una herramienta validada con preguntas preliminares para descartar el análisis de subgrupos sin condiciones mínimas relevantes, y una lista de verificación con recomendaciones de aplicabilidad.

Resultados. Se encontraron un total de 54 resultados y se seleccionaron cinco ECA. Según la primera metodología, sólo se encontró heterogeneidad consistente en el tiempo hasta la mejora clínica y la mejor puntuación del estado clínico en el día 15 para los pacientes con COVID-19 grave y <7 días de síntomas. Sobre la segunda metodología, estos resultados sobre el uso temprano de remdesivir pueden aplicarse a la práctica clínica con precaución.

Conclusiones. Se desarrolló una búsqueda sistemática y la aplicación de una metodología establecida para la interpretación del análisis de subgrupos sobre el uso temprano de remdesivir. Los resultados en la COVID-19 grave sugirieron que el uso temprano de remdesivir proporciona un mayor beneficio en <7 días de síntomas para el tiempo de mejora clínica y mejor puntuación del estado clínico en el día 15. Los estudios futuros podrían utilizar el corte de 7 días de síntomas para evaluar el remdesivir.

Palabras clave: COVID-19; remdesivir; toma de decisiones clínicas; análisis de subgrupos; evaluación de fármacos.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has affected 200 million people and it has leaded to more than four million of deaths worldwide [1]. Different drugs were used experimentally to treat this infection due to emergency situation and many studies were developed [2,3]. However, only vaccines and corticosteroids showed a clear relevant benefit [4-7].

Remdesivir is a prodrug with *in vitro* activity against severe acute respiratory syndrome coronavirus 2. Clinical results of these antiviral were evaluated in randomized clinical trials (RCT). Initially, Wang *et al* assessed remdesivir *versus* placebo including patients from Wuhan [8]. No statistically significant difference was observed for any endpoint analyzed in this RCT, such as time to clinical improvement and mortality at day 28. Despite this, a possible benefit in time to clinical improvement was suggested in time to clinical improvement for early-treated COVID-19 regarding a subgroup data.

Subgroup analysis compares the effect of a health intervention in different fractions of population. Interpretation of subgroup analysis presents limitations, so it should be conducted prudently and with strict methodological criteria [9]. Additional determinations and redistributions of patients for each characteristic subdividing population increase the possibility of obtaining differences by chance [10]. Distribution of population into subgroups reduces the statistical power of an analysis and the ability to detect differences between subgroups. Therefore, α and β errors are increased in subgroup analysis. Thus, misinterpretations often occur. Some of them include intragroup results assessment of hypothetical benefit for an intervention in a subgroup of patients without statistical difference with respect to complementary subgroup [11].

The commercialization of remdesivir and the health crisis due to COVID-19 triggered a barrage of hypotheses and claims. One of these hypotheses was directed to the timing of the antiviral use, associating the early treatment of remdesivir with greater benefit for patients [8,12]. However, was it statistically shown? About what endpoints? What is the real cut-off to differentiate early from late use of remdesivir? The answers to these questions are controversial and the use of the drug could significantly influence the efficiency in healthcare systems. A detailed assessment of this issue could prevent future mistakes in similar emergency situations. The objective of this study is the methodological interpretation of subgroup analyzes according to remdesivir treatment initiation with respect to symptom onset in patients with COVID-19, using established methodologies.

METHODS

Literature search. PICOS strategy was used to formulate the research question: population, intervention, comparator, outcome and study design. COVID-19 patients were included as population. Remdesivir treatment (or its combinations) was intervention. Any comparator was included. Outcome was subgroup analysis regarding the symptom onset for any endpoint. RCT was the selected study design.

Preferred Reporting Items for a Systematic Review and Meta-analysis (PRISMA) guidelines was applied in the systematic search. The review was conducted in Pubmed® database up to August 20, 2021. Search strategy was performed using screening tool "Clinical Queries/Narrow": *(remdesivir AND covid) AND (Therapy/Narrow[filter])*. Subsequently, a citation tracking was conducted.

Screening and selection of studies. Two investigators developed the search independently. Disagreements were resolved by discussion. Titles and abstracts of review records were checked to identify and discard studies without the established inclusion criteria. Full text of search results was examined in eligibility process. RCTs about COVID-19 with a subgroup analysis according to use of remdesivir with respect to symptom onset were selected. Early use of remdesivir was defined as the start of treatment before a certain day from symptom onset, which was detailed in each study. Late use of remdesivir occurred after that cut-off. Records obtained from the review in a language other than Spanish or English were excluded.

Data extraction. RCT included were analyzed in order of publication date. The following data were collected: authors, publication date, population, intervention and comparator therapies, sample sizes, endpoints, number of days subdividing the global population and efficacy of treatments.

Data analysis. Evaluation criteria of subgroup analysis from Sun *et al* were applied [9]: statistical interaction, pre-specification of subgroups, biological plausibility supporting the observed effect and consistency of subgroup results with other studies or within the same trial. Statistical interaction assesses whether difference among different subgroups is compatible with chance using the probability of interaction p(i). Considering the limitations of subset data, heterogeneity is defined as a statistically significant difference among subgroups [p(i)<0.1] [13]. Estimation of p(i) was obtained with calculators using relative risk values, odds ratio, hazard ratio and confidence intervals [14-19]. If insufficient data were provided, p(i) was evaluated using graphs when it was possible. Pre-specification of subgroups avoids the consideration of really non-existent differences caused by multiplicity. Biological plausibility evaluates the existence of hypotheses that justify differences between subgroups. Consistency assessed the agreement among subgroup data of similar studies or endpoints within the same RCT (internal consistency).

After that, a validated tool to assess the applicability of subgroup analysis was used [20]. It is a systematic methodology with two parts: preliminary questions to discard the assessment of subgroup results without relevant minimum conditions, and a checklist. Preliminary questions assess the evidence level of study, relevance of the endpoint evaluated, existence of a difference in effect [p(i)<0.1] among subgroups and temporal sequence between subpopulation generating

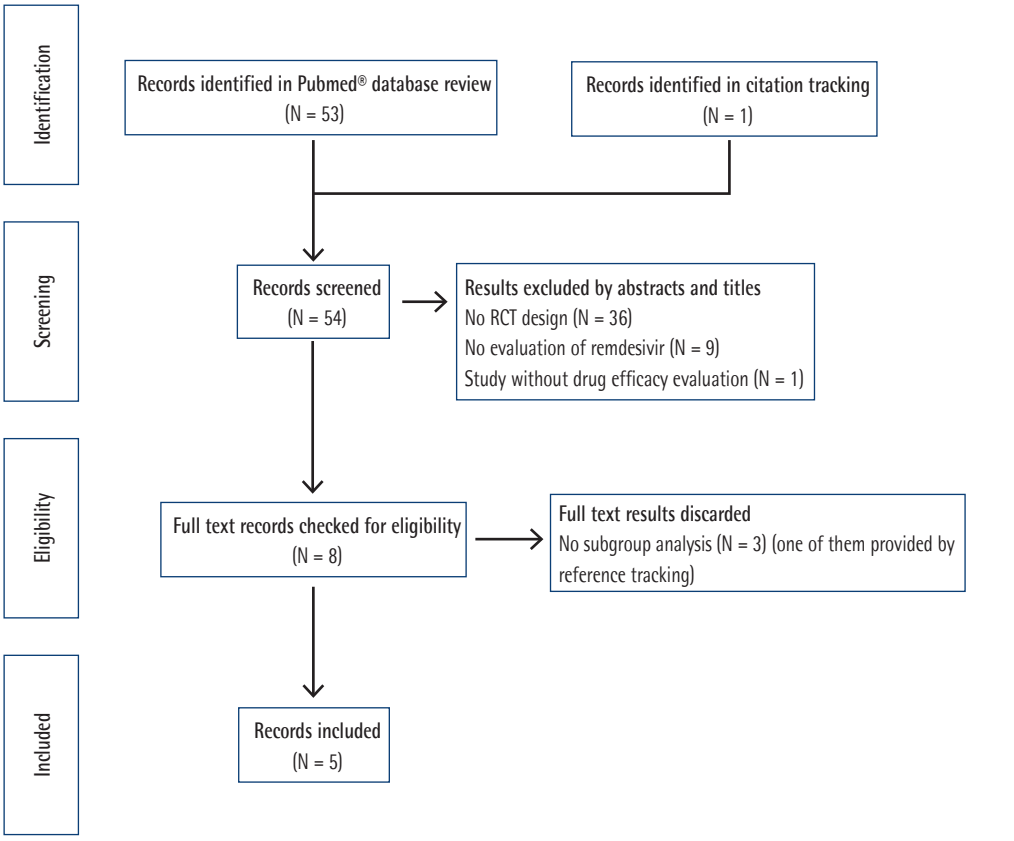

Figure 1 | **Review of literature**

factor and health intervention. A negative answer to any of preliminary questions discards subsequent questions, applicability of checklist and subgroup analysis. Checklist assigns an evaluation and score to a series of criteria for the interpretation of subgroup analysis: statistical association including p(i), pre-specification of subgroups, sample size, number of factors considered, and overall outcome of study; biological plausibility and consistency. Higher scores are related to higher reliability of each criterion: probable (+3 points), possible (+2), doubtful (0) and null (-3). Overall score is associated with a recommendation for applicability of results in clinical practice. Higher overall scores correspond to greater applicability and recommendations for consideration in clinical decision-making: 9-7 points (probable recommendation) mean applicability of subgroup results; 5-6 points (possible recommendation) indicate prudent applicability; 3-4 points (doubtful recommendation) mean avoid applicability with exceptions; and <3 points (null recommendation) indicate no application of subgroup data.

Risk of bias evaluation. RCT results may be affected by the prognosis of recruited patients. Disease severity, coexisting comorbidities and baseline score on ordinal scales about oxygen use of patients of RCTs were checked. Sample sizes of subgroups were evaluated.

RESULTS

Characteristics of studies. Fifty-three results were found in the systematic search. A RCT comparing 5-day and 10-day regimens of remdesivir without a subgroup analysis was found in citation tracking [21]. Exclusion criteria had the following distribution: 36 studies presented a design different from RCT design, 9 evaluated a drug other than remdesivir, 3 without subgroup analysis and one without efficacy consideration. Of the total of 54 records, 5 RCTs were included. Figure 1 illustrates the literature review regarding the PRISMA protocol and Table 1 shows data from included studies.

Subgroup analysis. Wang *et al* published a subgroup analysis regarding the effect of early and late use of remdesivir (with a cut-off for 10 days from symptom onset) for the following endpoints: time to clinical improvement, mortality at day 28 and viral RNA load on upper respiratory tract [8]. Criteria for interpretation of subgroup analysis described in Sun *et al* were applied [9]. P(i) was not detailed in the trial

Table 1 | **Data from randomized clinical trials included in study**

Authors	Online publication date	Population	Intervention	Comparator	Trial sample size	Endpoints with subgroup analysis	Subgroups according to days from symptom onset to treatment	Sample size of the early remdesivir use subgroup[a]	Sample size of the late remdesivir use subgroup[a]	Efficacy for endpoints in global population (95% CI)	Efficacy in subgroup with the early use of remdesivir (95% CI)	Efficacy in subgroup with the late use of remdesivir (95% CI)
Wang et al. [8]	April 29, 2020	Patients with severe COVID-19	Remdesivir 200 mg on day 1 and remdesivir 100 mg on days 2-10	Placebo	237	Time to clinical improvement	≤10 days and >10 days	118	115	Hazard Ratio: 1.23 (0.87 to 1.75)	Hazard Ratio: 1.52 (0.95 to 2.43)	Hazard Ratio: 1.07 (0.63 to 1.83)
						Mortality at day 28	≤10 days and >10 days	118	115	Rate differences: 1.1% (−8.1 to 10.3)	Rate differences: -3.6% (−16.2 to 8.9)	Rate differences: 4.6% (−8.2 to 17.4)
						Viral RNA load on upper respiratory tract	≤10 days and >10 days	64	60	No data of viral load −Log10 copies/mL (graphs only)	No data of viral load −Log10 copies/mL (graphs only)	No data of viral load −Log10 copies/mL (graphs only)
Spinner et al. [23]	August 21, 2020	Patients with moderate COVID-19	Remdesivir 200 mg on day 1 and remdesivir 100 mg on day 2-10 (5-days and 10-days course)	Standard of care	596	Clinical status on study day 11	5-days course: <9 days and ≥9 days	No data	No data	No data of difference in proportions (graphs only)	No data of difference in proportions (graphs only)	No data of differencein proportions (graphs only)
							10-days course: <9 days and ≥9 days	No data	No data	No data of difference in proportions (graphs only)	No data of difference in proportions (graphs only)	No data of difference in proportions (graphs only)
Beigel et al. (ACTT-1 Study Group, final report) [24]	October 9, 2020	Patients with severe COVID-19	Remdesivir 200 mg on day 1 and remdesivir 100 mg on days 2-10	Placebo	1062	Time to clinical improvement	≤10 days and >10 days	676	383	Rate ratio: 1.29 (1.12–1.49)	Rate ratio: 1.37 (1.14–1.64)	Rate ratio: 1.20 (0.94–1.52)
							≤9 days and >9 days	582	477	Rate ratio: 1.29 (1.12–1.49)	Rate ratio: 1.32 (1.09 to 1.61)	Rate ratio: 1.29 (1.04 to 1.59)
							1st Quartile: <7 days[b]	282	777	Rate ratio: 1.29 (1.12–1.49)	Rate ratio: 1.92 (1.41 to 2.60)	-
							2nd Quartile: 7 to ≤ 9 days[b]	300	-	Rate ratio: 1.29 (1.12–1.49)	Rate ratio: 0.99 (0.76 to 1.28)	-
							3th Quartile: 10 to ≤ 12 days[b]	221	-	Rate ratio: 1.29 (1.12–1.49)	Rate ratio: 1.45 (1.07 to 1.98)	-
							4th Quartile: ≥13 days[b]	803	256	Rate ratio: 1.29 (1.12–1.49)	Rate ratio: 1.15 (0.86 to 1.54)	-
						Better (Lower) clinical status score at day 15	≤10 days and >10 days	676	383	Odds ratio: 1.6 (1.3 to 1.9)	Odds ratio: 1.7 (1.3 to 2.2)	Odds ratio: 1.3 (0.9 to 1.9)
							≤9 days and >9 days	582	477	Odds ratio: 1.6 (1.3 to 1.9)	Odds ratio: 1.7 (1.2 to 2.2)	Odds ratio: 1.4 (1.0 to 1.9)
							1st Quartile: <7 days[b]	282	777	Odds ratio: 1.6 (1.3 to 1.9)	Odds ratio: 2.7 (1.8 to 4.2)	-
							2nd Quartile: 7 to ≤ 9 days[b]	300	-	Odds ratio: 1.6 (1.3 to 1.9)	Odds ratio: 1.1 (0.8 to 1.7)	-
							3th Quartile: 10 to ≤ 12 days[b]	221	-	Odds ratio: 1.6 (1.3 to 1.9)	Odds ratio: 1.4 (0.9 to 2.3)	-
							4th Quartile: ≥13 days[b]	803	256	Odds ratio: 1.6 (1.3 to 1.9)	Odds ratio: 1.3 (0.9 to 2.1)	-

Table 1 Data from randomized clinical trials included in study (cont.)

Authors	Online publication date	Population	Intervention	Comparator	Trial sample size	Endpoints with subgroup analysis	Subgroups according to days from symptom onset to treatment	Sample size of the early remdesivir use subgroup[a]	Sample size of the late remdesivir use subgroup[a]	Efficacy for endpoints in global population (95% CI)	Efficacy in subgroup with the early use of remdesivir (95% CI)	Efficacy in subgroup with the late use of remdesivir (95% CI)
Kalil et al. [25]	January 5, 2021	Patients with severe and moderate COVID-19	Remdesivir 200 mg on day 1 and remdesivir 100 mg on days 2-10 + baricitinib 4 mg for 14 days	Placebo + Remdesivir 200 mg on day 1 and remdesivir 100 mg on days 2-10	1033	Time to clinical improvement	≤10 days and >10 days	764	253	Rate ratio: 1.16 (1.01–1.32)	Rate ratio: 1.13 (CI 0.97–1.32)	Rate ratio: 1.27 (0.97–1.67)
Barratt-Due et al. [26]	July 13, 2021	Patients with severe and moderate COVID-19	Remdesivir 200 mg on day 1 and remdesivir 100 mg until day 9	Standard of care	185	Oropharyngeal viral clearance	<7 days and ≥7 days	No data	No data	Difference in daily decrease rate: 0.113 (-0.001 to 0.227)	Difference in daily decrease rate: 0.19 (0.03 to 0.36)	Difference in daily decrease rate: 0.02 (-0.15 to 0.19)

[a]The early use of remdesivir was associated with the subgroup of patients who received remdesivir with the lowest number of days between the onset of symptoms and the start of treatment. The late use of remdesivir is the subgroup of patients with the highest number of days in this period of time.

[b]Quartiles about duration of symptoms prior to enrollment of patients in ACTT-1 Study Group (final report) only presented one efficacy data. Complementary subgroup in first and fourth quartiles was estimated from trial data.

Table 2 **Summary of interpretation for subgroup analysis from studies.**

Methodology	Criteria		Wang et al. [8] Endpoints			Spinner et al. [23] Endpoints		Beigel et al (ACTT-1 Study Group) [24] Endpoints for 7 days of symptons cut-off		Kalil et al. [25] Endpoints	Barratt-Due et al. [26] Endpoints
			Time to clinical improvement	Mortality at day 28	Viral load on upper respiratory tract	Clinical status on study day 11		Time to clinical improvement	Better clinical status score at day 15	Time to clinical improvement	Oropharyngeal viral clearance
						5-day course of remdesivir	10-day course of remdesivir				
Sun et al. [9]	Statistical interaction		No	No	Insufficient data	No	Yes	Yes	Yes	No	No
	Pre-specification of analysis		Undefined	Undefined	Undefined	No	No	Yes	Yes	Yes	Yes
	Biological plausibility		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Consistency of subgroup results		Results with internal consistency of no benefit	Results with internal consistency of no benefit	Insufficient data	No	No	Yes (internal consistency of greater benefit in early use of remdesivir)	Yes (internal consistency of greater benefit in early use of remdesivir)	Yes (consistency of lack of differences between early and late use of remdesivir)	No
Validated tool (Gil-Sierra et al.) [20]	Preliminary questions	The study shows the highest level of evidence with subset analysis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
		Clear clinical relevance of considered endpoint or primary surrogate outcome of study	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No
		Existence of difference in effect between the subgroups for the factor evaluated ($p(i) < 0.1$)	No	No	Not applied	No	Yes	Yes	Yes	No	Not applied
		Determining factor of subgroup analysis was present prior to health intervention	Not applied	Not applied	Not applied	Not applied	Yes	Yes	Yes	Not applied	Not applied
	Checklist Statistical association (score) Biological plausibility (score) Consistency (score) Recommendation (sum)		Not applied	Not applied	Not applied	Not applied	Applied Null (-3 points) Probable (+3 points) Null (-3 points) Null (-3 points): "Subgroup analysis should not be considered"	Applied Probable (+3 points) Probable (+3 points) Doubtful (0 points) Possible (6 points): "Subgroup results analysis may be applied to clinical practice with caution"	Applied Probable (+3 points) Probable (+3 points) Doubtful (0 points) Possible (6 points): "Subgroup results analysis may be applied to clinical practice with caution"	Not applied	Not applied

Table 3 **Data about benefit-related factors of patients in studies.**

Prognosis-related factors of patients	Wang et al. [8]	Spinner et al.[a] [23]	Beigel et al. (ACTT-1 Study Group) [24]	Kalil et al. [25]	Barratt-Due et al. [26]
Disease severity (%)					Moderate and severe
Moderate	0%	100%	9.9%	31.7%	Insufficient data
Severe	100%	0%	90.1%	68.3%	Insufficient data
Coexisting comorbidities (%)	71%	Insufficient data	81.7%-82.2%	87.1%-81.7%	Insufficient data
Hypertension	46%-38%	44%-43%-41%	50.6%-50.9%	51%-52%	36.6%-24.6%
Diabetes	25%-21%	44%-37%-38%	32%-31%	40%-36%	22%-15.8%
Cardiovascular disease	9%-3%[b]	58%-58%-54%	18%-16%	21%-21%	14.6%-21.1%
Obesity	Insufficient data	Insufficient data	46%-45%	58%-53%	28.9%-18.4%
Baseline score on ordinal scales about oxygen use (%)					
Hospitalized, requiring supplemental oxygen	82%-83%	12%-16%-19%	42.9%-39%	55.9%-53.3%	Insufficient data
Hospitalized, receiving non-invasive ventilation or high-flow oxygen devices	18%-12%	0%	17.6%-18.8%	20%-21.8%	Insufficient data
Hospitalized, receiving invasive mechanical ventilation or extracorporeal membrane oxygenation	0%-1%	0%	24.2%-29.6%	10.5%-11%	Insufficient data
Ordinal scale	Six-category scale	Seven-category scale	Eight-category scale	Eight-category scale	Ten-category scale

Data with one result details the percentage of global population in study. Data with two results represents the percentages collected from intervention and control arms (% of patients in intervention arm-% of patients in control arm). [a]Spinner et al presented three arms [23]: 10-day course of remdesivir, 5-day course of remdesivir and control arm (% of patients in 10-day remdesivir arm-% of patients in 5-day remdesivir arm-% of patients in control arm). [b]Only coronary heart disease.

for any endpoint. It was calculated and no heterogeneity was found between subgroups of early and late use of remdesivir for time to clinical improvement [p(i)=0.33] and mortality at day 28 [p(i)=0.37]. Insufficient data were provided to calculate p(i) between subgroups for viral RNA load on upper respiratory tract. Wang *et al* commented that no statistically significant difference was found for viral RNA load in upper respiratory tract between control and intervention arms in early and late use of remdesivir without mentioning p(i). Pre-specification of subgroup analyses was not defined in study protocol for any outcome. Biological plausibility supporting the early use of remdesivir in patients with COVID-19 appeared reasonable. This hypothesis was based on experience with viruses such as influenza or other pathogens [12,22]. Wang *et al* study was the first RCT about use of remdesivir in COVID-19 and consistency with previous trials could not be assessed. However, absence of heterogeneity between subgroups for time to clinical improvement and mortality at day 28 showed internal consistency of subgroup analysis [8].

Subsequently, the validated tool about applicability of subgroup analysis was used [20]. Some of preliminary questions were answered negatively in the three endpoints so checklist was not developed. Subgroup analysis regarding time of drug administration for time to clinical improvement and mortality at day 28 presented no differences of effect among subgroups [p(i)>0.1]. Insufficient data were provided for heterogeneity estimation for viral RNA load on upper respiratory tract. Likewise, it is a surrogate endpoint with little clinical relevance. Table 2 shows a summary of the interpretation for subgroup analysis described in Wang *et al* [8].

Spinner *et al* provided a trial with subgroup analysis based on early and late use of remdesivir (with a cut-off for 9 day) for clinical status on study day 11, for both 5-day and 10-day courses of remdesivir [23]. At first, criteria for subgroup analysis interpretation of Sun *et al* were used [9]. P(i) value was not described for clinical status on day 11 and no exact data were reported. However, forest plot in subset analysis showed almost total overlap between subgroups of early and late use of remdesivir for 5-day course [p(i)>0.2], while overlap was less than 50% for 10-day course. Difference in proportions and 95% confidence intervals (95%CI) were extracted from graphical representations of forest plot for 10-day scheme. Difference in proportions in early use of remdesivir (<9 days) was 12.6 (95%CI, -0.6 to 5.3) and late use of remdesivir (≥9 days) was -4 (95%CI, -18.6 to 10). P(i) between these subgroups was estimated as 0.0393. Subgroup analysis about time of remdesivir use with respect symptom onset was not prespecified (*post hoc* analysis). Biological plausibility supported early use of remdesivir, in accordance with previous experience with viruses and other microorganisms [12,22]. There was internal inconsistency between subgroup results of 5-day and 10-day courses of treatment within the same trial [23]. One remdesivir scheme proved a statistically significant difference between subgroups according to timing of remdesivir use and the other scheme did not. Different cut-off was evaluated in Wang *et al* (10 days), wich showed no differences in effect between subgroups [8]. No comparisons can be performed among subgroups with different time periods.

Validated tool about applicability of subgroup analysis was applied [20]. For 5-day course of remdesivir, preliminary question regarding the existence of differences in effect between subgroups was answered negatively for clinical status on study day 11 [p(i)>0.2]. For 10-day course of remdesivir, all preliminary questions were answered positively. When applying the checklist, statistical association presented "null" evaluation (-3 points) due to lack of pre-specification of the considered factor (*post hoc* analysis). Biological plausibility had "probable" assessment (+3 points) and consistency was "null" (-3 points). The sum of these scores (-3 points) was associated with "null" recommendation for applicability in clinical practice of subset results. Therefore, results of early use subgroup (<9 days) from 10-day regimen of remdesivir should not be considered for decision-making in moderate COVID-19. A summary of interpretation about subgroup analyzes described in Spinner *et al* can be consulted in Table 2.

Beigel *et al* (final report of ACTT-1 Study Group) published a subgroup analysis regarding duration of symptoms prior to enrollment for two endpoints: time to clinical improvement and better clinical status score at day 15 [24]. Subset analyses evaluated these efficacy endpoints across following cuts-off: quartiles of days (<7 days, 7 to ≤9 days, 10 to ≤ 12 Days, >13 Days); ≤9 days *versus* >9, and ≤10 days *versus* >10. Criteria of Sun *et al* were used to analyze subset analysis [9]. No p(i) value was reported for outcomes in different cut-offs. It was calculated and heterogeneity [p(i)=0.009] was found in the first quartile of days (subgroup with <7 days of symptoms before enrollment *versus* ≥7 days) for time to clinical improvement. P(i)<0.05 was also estimated in the first quartile for better clinical status score at day 15. However, a value of P(i)>0.2 was quantified at any of other cut-offs for both endpoints. Subgroup analysis about duration of symptoms before to enrollment was pre-specified in trial protocol. Biological plausibility justified early use of remdesivir according to experience with other microorganisms [12, 22]. Internal consistency was found between subgroup results for time to clinical improvement and better clinical status score at day 15. Both endpoints presented statistically significant improvement [p(i)<0.05] for patients with early treatment of remdesivir in <7 days of symptoms. On the other hand, results at cuts-off for 9 and 10 days were compared with previously published data [8, 23]. None of these studies showed consistent benefit for early use of remdesivir at the day 9 and 10 thresholds. The rest of subgroups (7 to ≤9 days, 10 to ≤ 12 Days and >13 Days) were not considered in previous studies and therefore could not be compared with Beigel *et al* [24].

The tool about applicability of subgroup analysis was used [20]. Preliminary questions were answered positively in the first quartile (<7 days of symptoms before enrollment *versus* ≥7 days) for time to clinical improvement and better clinical status score at day 15. When applying the checklist, statistical association showed "probable" consideration (+3 points). Biological plausibility also presented "probable" assessment (+3 points). Consistency had "doubtful" evaluation (0 points). The sum of scores (+6 points) was related with "possible" recommendation for applicability in clinical practice of subgroup results. Thus, a greater benefit of early use of remdesivir in <7 days of symptoms for time to clinical improvement and better clinical status score at day 15 may be applied to clinical practice with caution in severe COVID-19. Preliminary questions about the existence of differences in effect among subgroups were answered negatively for the rest of cut-offs in selected endpoints. A summary of subgroup analysis interpretation about 7 days of symptoms cut-off in final report of ACTT-1 Study Group is shown in Table 2.

Kalil *et al* compared baricitinib plus remdesivir *versus* remdesivir [25]. This RCT published a subgroup analysis regarding symptom onset (with a cut-off for 10 days) for time to clinical improvement. Subgroup analysis criteria of Sun *et al* were applied [9]. No p(i) estimation was presented for the evaluated endpoint. P(i) was calculated and no heterogeneity was observed between subgroups of early and late use of antiviral drug [p(i)=0.46]. Subgroup analysis regarding to duration of symptoms was pre-specified in trial protocol. Biological plausibility justifying early use of remdesivir was based on previous experience with viruses and other microorganisms [12,22]. Prior studies on the use of remdesivir in COVID-19 containing subgroup analysis according to symptom onset (with a cut-off for 10 days) showed no heterogeneity of the antiviral effect [8,24]. These data could be consistent with subgroup results reported in Kalil *et al* [25].

Afterwards, validated clinical applicability tool for subgroup analysis was applied [20]. Preliminary question about the existence of differences in effect among subgroup according to early or late use of remdesivir was answered negatively [p(i)>0.1]. Therefore, the rest of the tool was not applied. Table 2 describes a summary of interpretation about subgroup analysis presented in Kalil *et al*.

Barratt-Due *et al* presented a subset analysis about the time of use of remdesivir respect to symptom onset (with a cut-off for 7 days) for oropharyngeal viral clearance [26]. Sun *et al* criteria for interpretation of subgroup analysis were applied [9]. P(i) was not reported in the trial for oropharyngeal viral clearance. Own estimations showed no heterogeneity between subsets of early and late use of remdesivir for the selected endpoint [p(i)=0.166]. Pre-specification of subset analysis according to symptom onset was defined in protocol. Biological plausibility of early use of remdesivir for COVID-19 was justified by experience with other infectious pathogens [12,22]. The absence heterogeneity between subsets of early and late use of remdesivir in the cut-off for 7 days of Barratt-Due *et al* was no consistent compared to statistically significant differences observed in Beigel *et al* [24,26].

Thereupon, the validated tool about subset analysis was used [20]. Preliminary question on the relevance of the endpoint evaluated in selected subgroups was answered negatively, so the rest of the tool was not applied. A summary of interpretation about subset analysis reported in Barratt-Due *et al* is detailed in Table 2.

Risk of bias evaluation. Three RCTs included a popula-

tion with moderate and severe COVID-19, one RCT evaluated only patients with moderate COVID-19 and other assessed only patients with severe disease. The percentage of patients with comorbidities in the RCTs included in this study ranged from 71% to 87.1%. Hypertension was the most frequently recorded comorbidity in all RCTs (24.6% to 52%). Baseline score on ordinal scales about oxygen use of patients presented worse results in Beigel *et al* and Kalil *et al* [24,25], where percentage of patients with high-flow oxygen devices or non-invasive ventilation was 17.6% to 21.8% and patients with invasive mechanical ventilation or extracorporeal membrane oxygenation ranged from 10.5% to 29.6%. RCTs used different ordinal scales: one study applied a six-category ordinal scale, one evaluated patients with a seven-category scale, two RCTs considered an eight-category scale and one applied a ten-category scale. Data about prognosis-related factors of patients were detailed in Table 3.

Wang *et al* assessed more than 200 patients in subgroup analysis regarding time of antiviral drug for time to clinical improvement and mortality at day 28; and less than 130 patients for viral RNA load on upper respiratory tract [8]. More than 1,000 patients were included in subgroup analysis of Beigel *et al* and Kalil *et al* for time to clinical improvement and better clinical status score at day 15 [24,25]. The rest of endpoints in RCTs presented insufficient data about sample sizes of subsets. Table 1 provides the number of patients involved in subgroup analysis.

DISCUSSION

Results in patients with severe COVID-19 suggested that early use of remdesivir provides benefit compared to late use only in time to clinical improvement and better clinical status score at day 15 [24]. The effect of remdesivir in these endpoints has been statistically superior in patients with <7 days of symptoms. However, Barratt-Due *et al* found no differences between early and late use of remdesivir at 7 days cut-off for oropharyngeal viral clearance [26]. Oropharyngeal viral clearance is a subrogate endpoint with little clinical effect. It is a local measure and severe COVID-19 is a systemic disease with mainly pulmonary involvement. This could be an explanation for inconsistency of early and late subgroup results at 7 days cut-off presented in Barratt-Due *et al* and Beigel *et al* [24]. On the other hand, no greater effect of early use of remdesivir was observed for other cut-offs and outcomes. Thus, previous experience of greater benefit from early neuraminidase treatment in patients with influenza infection was partially confirmed in early use of remdesivir against COVID-19 [27], since benefit was observed in outcomes with limited clinical relevance.

The results of our study are complementary to WHO Solidarity trial data [28], which is the most important study on the use of COVID-19 treatments. Authors of WHO Solidarity trial concluded that remdesivir showed little or no effect on hospitalized patients with COVID-19, according to overall mortality, time of hospitalization and initiation of ventilation. This study was not included in our review due to lack of subset analysis of remdesivir effect regarding the time of symptom onset.

For cut-off of 9 days of symptoms, our review found apparent heterogeneity between subgroups of 10-day course of remdesivir for clinical status on day 11 in moderate COVID-19 trial of Spinner *et al* [23]. These differences among subsets regarding symptom onset were calculated from graphical representations -without exact data provided by authors-. Furthermore, *post hoc* nature of this subset analysis should not be forgotten. Likewise, Spinner *et al* and other similar studies reported no differences between subgroups of early and late use of 5-day remdesivir regimen for cut-off of 9 days of symptoms [23,24]. The apparent differences between subgroups in 10-day course of remdesivir can be attributed to a multiplicity of determinations and limitations of subgroups [10].

Subgroup analyses should be considered with caution due to their limitations [9,10,29]. Wang *et al* committed one of the most frequent methodological errors. They considered intragroup differences between remdesivir and placebo in early treatment subset for time to clinical improvement and viral load in upper respiratory tract at 10-day cut-off [8,15,30,31]. No interaction test was calculated with complementary subset. Numerical differences in time to clinical improvement favorable to early use of remdesivir was highlighted, facilitating an inadequate interpretation of subgroup analysis that may influence clinical decision-making. In addition, differences of effect observed in a subset analysis should not always apply. First, clinical relevance of endpoints needs to be evaluated. Both viral load and time to clinical improvement can be considered as measures of little clinical relevance if they are not related with a reduction in mortality.

Our review found patients with different characteristics in RCTs about the use of remdesivir in COVID-19 [8,23-26]. There were studies with only moderate or severe COVID-19 and others recruited both populations. Variability was also observed in oxygenation and ventilation for COVID-19 patients in RCTs. Beigel *et al* and Kalil *et al* presented the worst results of baseline score on ordinal scales about oxygen use [24,25]. Further, patients were evaluated using different scales. On the other hand, Beigel *et al* and Kalil *et al* developed subgroup analysis regarding the timing of remdesivir with the largest sample sizes. Generally, studies did not provide all the essential information for the subset data.

This work is an illustrative example of how a methodological assessment of subset analysis can avoid making premature statements. Two previous publications were used. Sun *et al* established an adequate basis for the assessment of subgroup analyzes, but inexperienced evaluators may doubt the importance or order about interpretation criteria [9]. This possible limitation can be minimized by validated tool of Gil-Sierra *et al* [20], that also values additional considerations with respect to the first methodology.

Although many drugs –such as lopinavir/ritonavir or hydroxychloroquine- have been widely used, only glucocorticoids and vaccines showed a clear reduction of mortality in COV-

ID-19 [3, 4, 32,33]. Uncertainty about the effect of therapeutic alternatives is still high [5]. The development of systematic methodologies to evaluate new scientific evidence is necessary to reduce superfluous or negative effects of drugs -and unnecessary expenses- in patients with COVID-19. Minimum costs of production for remdesivir have been estimated at US $0.93/day [34]. However, remdesivir acquisition price is much higher than costs of production. Therefore, optimization of the use of this antiviral is very important for efficiency of health systems due to its important economic impact.

CONCLUSION

We conducted a study with a systematic search and application of an established methodology for interpretation of subgroup analysis about early use of remdesivir in COVID-19. Moreover, our review detailed essential estimates for interpretation of subset analyzes not previously described. This work found a statistically significant superior benefit of early use of remdesivir for patients with severe COVID-19 and <7 days of symptoms for time to clinical improvement and better clinical status score at day 15. No greater benefit was associated with early use of remdesivir in other outcomes or time cuts. Finally, it seems reasonable to apply the 7-day cut-off from symptoms onset to evaluate the early use of remdesivir for COVID-19 in future studies.

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CONFLICTS OF INTEREST

Manuel David Gil-Sierra: membership of an advisory board (consultation fees), lectures for Janssen Pharmaceutica and Pfizer (reimbursement for attending symposia) of another cancer drugs. The rest of the authors have no conflict of interest to declare.

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Estudio observacional retrospectivo de persistencia de infección por SARS-CoV-2 en pacientes tratados previamente con rituximab

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RESUMEN

Introducción. La inmunodepresión inducida por rituximab podría ser un factor de riesgo de mortalidad por COVID-19. El objetivo del estudio fue describir la prevalencia de infección por SARS-CoV-2 en pacientes que habían recibido rituximab y conocer si conduce a una mayor persistencia del virus.

Material y métodos. Estudio observacional retrospectivo de pacientes que recibieron rituximab en los 6 meses previos al inicio de la pandemia, analizándose la presencia de infección. Se recogieron las siguientes variables: edad, sexo, enfermedades previas, factores de riesgo para COVID-19, dosis recibidas de rituximab, resultados de los test diagnósticos, hospitalización, tipo de soporte ventilatorio, desarrollo de eventos tromboembólicos y tratamiento recibido. Se realizó un análisis descriptivo de todas las variables y se compararon pacientes que se habían infectado (C+) y los que no (C-).

Resultados. 68 pacientes habían recibido rituximab (mediana de dosis acumulada: 4.161mg (2.611–8.187,5)), 54,4% hombres con edad media de 60,8 años (15,7; 25-87). Se confirmó C+ en 22 pacientes, entre los cuales existían los siguientes antecedentes: 45,5% hipertensión arterial, 36,4% Diabetes Mellitus, 31,8% tabaquismo/exfumador, 22,7% neumopatía, 13,6% cardiopatía y 4,5% obesidad. No se apreciaron diferencias estadísticamente significativas entre C+ y C-. Sólo 2 pacientes C+ desarrollaron inmunidad y 10 de ellos (45,5%) no negativizaron PCR a la finalización del seguimiento. No se encontró asociación con la dosis acumulada de rituximab. La tasa de mortalidad en la C+ fue de 22,7%.

Conclusiones. En nuestros pacientes tratados con rituximab y con infección por SARS-CoV2 se observó una peor evolución y una mayor persistencia de la infección, por lo que debería valorarse el uso de otras alternativas durante la pandemia, ya que la disminución de la función de células B podría producir un mayor riesgo de evolución fatal por COVID-19.

Palabras clave: SARS-CoV-2, Rituximab, Persistencia

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Retrospective observational study of the persistence of SARS-CoV-2 infection in patients previously treated with rituximab

ABSTRACT

Introduction. Rituximab-induced immunosuppression could be a risk factor for mortality from COVID-19. The aim of the study was to describe the prevalence of SARS-CoV-2 infection in patients who have received rituximab and its association with a persistent viral infection

Material and methods. Retrospective observational study of patients who received rituximab in the 6 months before to the onset of the pandemic. We analyzed the presence of infection and associated them with demographic variables, pathological history related to an increased risk of developing severe COVID-19, the doses of rituximab received, the type of ventilatory support, thromboembolic events, and the treatment received. A descriptive analysis of all the variables was carried out and infected and uninfected patients were compared.

Results. We screened a total of 68 patients who had received rituximab (median cumulative dose: 4,161mg (2,611–8,187.5)). 54.4% men, mean age 60.8 years (15.7; 25-87)). C + was confirmed for 22 patients. Of these, 45.5% had high blood pressure, 36.4% Diabetes Mellitus, 31.8% smokers/ex-smoker, 22.7% lung disease, 13.6% heart disease and 4.5% obesity. There were no statistically significant differences between C+ and C-. Only 2 patients developed immunity. For 10 patients (45.5%) did not have a negative CRP until the end of the follow-up. There was no association with cumulative dose of rituximab. The mortality rate was 22.7% in the C+.

Conclusions. We observe that the persistence of the infection leads to a worse evolution of COVID-19. The use of alternatives should be considered during the pandemic, because of patients with decreased B-cell function may have high risk of fatal progression from COVID-19.

Keywords: SARS-CoV-2, Rituximab, Persistence

INTRODUCCIÓN

Desde la llegada de la pandemia producida por el virus SARS-CoV-2, la comunidad científica aúna sus esfuerzos para intentar dilucidar en tiempo récord toda la información posible sobre este virus y frenar su avance.

Muchos estudios alrededor del mundo ya sugieren que la edad y las enfermedades crónicas (especialmente las pulmonares) aumentan el riesgo de desarrollar neumonía relacionada con el SARS-CoV-2 (COVID-19) y fallo respiratorio asociado [1]. Por otro lado, se presume que los pacientes inmunodeprimidos tienen un riesgo de enfermedad más grave, pero a la vez se considera que pueden tener una disminución de la respuesta inflamatoria, la cual causa gran parte del daño en esta enfermedad. Se asume por tanto que los pacientes con cáncer y los receptores de un trasplante de órgano sólido pueden tener un mayor riesgo de enfermedad grave por COVID-19 y muerte, mientras que, para aquellos con otros tipos de inmunodepresión, la evidencia actual es menos clara [2]. De aquí que, si el tratamiento inmunosupresor, el cual comprende fármacos muy diferentes, es un factor de riesgo o protector, aún no ha sido esclarecido hasta la fecha. Los datos provenientes de otras infecciones virales más antiguas como el virus de la gripe han demostrado un mayor riesgo de infección grave en estos pacientes inmunocomprometidos, pero otros datos provenientes de la mortalidad ocasionada por otros coronavirus establecen que la sola presencia de terapia inmunosupresora no determina un peor pronóstico [3].

La observación clínica hace sospechar que la inmunodepresión inducida por rituximab es un factor de riesgo de morbilidad y mortalidad en la infección por el SARS-CoV-2. Además, se desconoce el tratamiento óptimo de estos pacientes en los que se observa una infección persistente aparentemente ligada al tratamiento previo con este fármaco.

Rituximab es un anticuerpo monoclonal que se une específicamente al antígeno CD20, expresado en los linfocitos pre-B y B maduros. Tras completarse la administración de la primera dosis, los recuentos de células B periféricas disminuyen por debajo de lo normal, y el periodo de recuperación oscila entre 6 y 12 meses tras la finalización del tratamiento, aunque puede ser superior en algunos pacientes e incluso resultar tras la administración de una sola dosis [4].

En el caso de la infección por SARS-CoV-2, la inmunidad innata puede ser capaz de controlar la enfermedad, y, si el virus supera este primer sistema de control, la respuesta inmune adaptada, mediada por anticuerpos, puede controlar su proliferación. Esta respuesta humoral parece ser crítica en la eliminación de las infecciones virales y en la prevención de la reinfección [5]. Los linfocitos T y B por tanto juegan un papel central en esta fase, conduciendo finalmente a la producción de anticuerpos con especificidad antiviral. Los nuevos coronavirus (SARS-CoV, MERS y SARS-CoV2) pueden, sin embargo, escapar a este mecanismo de control a través de la inducción de la apoptosis de linfocitos T [6].

Así, esa depleción prolongada de linfocitos B conlleva una respuesta inmune humoral deficiente frente a nuevos antígenos, pudiendo ser la causa de que se prolongue durante semanas o meses, lo que incrementa el riesgo de morbilidad y mortalidad para el paciente.

Hasta la fecha se desconoce el tratamiento óptimo de estos pacientes y cómo mejorar las probabilidades de éxito en su evolución.

Los objetivos del estudio fueron: i) describir la prevalencia de infección por SARS-CoV-2 en pacientes que previamente habían recibido rituximab; ii) conocer si la infección conduce a una mayor persistencia del virus y peor evolución debido a la inmunosupresión provocada por este fármaco y iii) evaluar la respuesta a los diferentes tratamientos recibidos utilizados contra el SARS-CoV-2 y sus manifestaciones clínicas.

MATERIAL Y MÉTODOS

Estudio observacional retrospectivo de los pacientes de un Hospital General que recibieron rituximab en los 6 meses previos al inicio de la pandemia (octubre 2019) hasta enero de 2021.

De los pacientes localizados, se analizó la presencia de infección confirmada por el virus SARS-CoV-2 durante el mismo periodo a través de la historia clínica electrónica para analizar su evolución y tratamiento.

La presencia de infección por SARS-CoV-2 fue confirmada mediante reacción en cadena de la polimerasa (PCR) o test de antígeno.

Se recogieron las siguientes variables de interés para conocer la evolución y el desarrollo de complicaciones relacionadas con la COVID-19:

- Edad, sexo, enfermedades previas y factores de riesgo para COVID-19 (hipertensión arterial (HTA), diabetes mellitus (DM), tabaquismo, neumopatía, cardiopatía y obesidad).
- Dosis previas recibidas de rituximab (dosis acumuladas y tiempo desde la última dosis recibida).
- Datos analíticos de infección por SARS-CoV-2: tipos de test realizados y su resultado.
- Evolución de la COVID-19: negativización de la PCR y formación de anticuerpos, desarrollo de neumonía, necesidad de hospitalización, tipo de soporte ventilatorio recibido, estancia en UCI, aparición de eventos tromboembólicos, tratamiento farmacológico recibido durante el ingreso o de forma ambulatoria, y administración de plasma hiperinmune de donante convaleciente. Entre los tratamientos considerados específicos para COVID-19 se consideraron: hidroxicloroquina, azitromicina, corticoides, remdesivir y tocilizumab. Se tuvieron en cuenta el resto de tratamiento utilizados como los antibióticos, N-acetilcisteína, atorvastatina, heparinas de bajo peso molecular y antiretrovirales, utilizados según la evidencia disponible en el momento.

La evolución de los pacientes fue evaluada según la clasificación de progresión de la enfermedad propuesta por la organización mundial de la salud (OMS) [7], recogiendo tam-

Tabla 1 **Análisis descriptivo.**

		Global (n = 68)	COVID-19 (n = 22)	COVID-19 (n = 46)	p
Edad media (DE; rango)		60,8 (15,7; 25 – 87)	57,3 (15,9; 25 – 80)	62,4 (15,6; 30 -87)	0,234
Sexo	Hombre	37 (54,4%)	10 (54,5%)	27 (58,7%)	0,305
	Mujer	31 (45,6%)	12 (45,5%)	19 (41,3%)	
Mediana última dosis rituximab (mg) (RIC)		676,5 (577 - 750)	615 (191,5)	681 (157)	0,359
Mediana dosis acumulada rituximab (mg) (RIC)		4161 (2611 - 8187,5)	4058,6 (3712,5)	4360 (6239)	0,159
Comorbilidades	HTA	27 (39,7%)	10 (45,5%)	17 (37%)	0,503
	DM	16 (23,5%)	8 (36,4%)	8 (17,4%)	0,084
	Tabaquismo	15 (22,1%)	7 (31,8%)	8 (17,4%)	0,218
	Neumopatía	9 (13,2%)	5 (22,7%)	5 (8,7%)	0,136
	Cardiopatía	12 (17,6%)	3 (13,6%)	9 (19,6%)	0,738
	Obesidad	8 (11,8%)	1 (4,5%)	7 (15,2%)	0,260

Tabla 2 **Diagnóstico para recibir rituximab**

	Global (n = 68)	COVID-19 (n = 22)	COVID-19 (n = 46)	p
Linfoma	38 (55,9 %)	14 (63,6%)	24 (52,2%)	
Enfermedad reumatológica	8 (11,8%)	3 (13,6%)	5 (10,9%)	
Enfermedad tejido conectivo	7 (10,3%)	3 (13,6%)	4 (8,7%)	
Enfermedad neurológica Inmunomediada	5 (7,4%)	0	5 (10,9%)	
Vasculitis	4 (5,9%)	1 (4,5%)	3 (6,5%)	
Síndrome antisintetasa	1 (1,5%)	0	1 (2,2%)	0,651
Púrpura trombocitopénica inmune	1 (1,5%)	0	1 (2,2%)	
Leucemia linfática crónica B	1 (1,5%)	0	1 (2,2%)	
Leucemia linfática crónica	1 (1,5%)	0	1 (2,2%)	
Granulomatosis de Wegener	1 (1,5%)	1 (4,5%)	0	
Glomerulonefritis	1 (1,5%)	0	1 (2,2%)	

bién si el paciente recibió el alta hospitalaria, sufrió reingresos o éxitus.

Se realizó un análisis descriptivo de todas las variables, media (desviación estándar) o mediana (rango intercuartílico) para variables cuantitativas y frecuencias absolutas y relativas para variable cualitativas. La comparación entre pacientes con infección positiva por SARS-CoV-2 (C+) y negativa (C-) se realizó mediante el test χ^2 o exacto de Fisher para variables cualitativas y el test t de Student o U-Mann Whitney para variables cuantitativas. Todos los cálculos se realizaron mediante el programa SPSSv18 y se tomó un valor de $p<0,05$ como estadísticamente significativo.

RESULTADOS

Se localizaron 68 pacientes que habían recibido rituximab durante el periodo establecido con una mediana de dosis acumulada de 4.161mg (2.611–8.187,5). El 54,4% eran hombres, con una edad media (DE; rango) de 60,8 años (15,7; 25 – 87) (Tabla 1). El diagnóstico predominante por el que recibieron rituximab fue linfoma (56%), seguido de patología reumatológica (32,1%) (Tabla 2).

Se confirmó C+ en 22 pacientes (mediante PCR+ en el 100%). De los C-, el 65,2% también tuvieron PCR u otra prueba

Tabla 3 Clasificación de la gravedad de la enfermedad según la OMS

OMS	COVID-19 (n = 22)
1	4 (18,2%)
2	1 (4,5%)
3	0
4	5 (22,7%)
5	4 (18,2%)
6	3 (13,6%)
7	0
8	0
9	0
10	5 (22,7%)

realizada negativa, no realizándose en el resto de C- por no presentar síntomas sugestivos de COVID-19 o visitas hospitalarias. La mediana de tiempo desde el último tratamiento en la cohorte C+ fue de 2,6 meses (RIC: 0,9 – 6,67).

En cuanto a factores de riesgo de mal pronóstico para padecer COVID-19 grave, de la cohorte C+; el 45,5% tenía HTA, 36,4% DM, 31,8% tabaquismo/exfumador, 22,7% neumopatía, 13,6% cardiopatía y 4,5% obesidad. No se apreciaron diferencias estadísticamente significativas entre la cohorte C+ y C- respecto a estos factores.

El 81,8% de los C+ fueron ingresados, con un tiempo mediano de hospitalización de 12 días (RIC: 0–200). El 50% precisó como mínimo gafas nasales y el 96,4% recibió tratamiento considerado específico para COVID-19. Un 22,7% (5 pacientes) fueron tratados con plasma hiperinmune como última opción ante la falta de aclaramiento viral, falleciendo 3 de ellos. Dos pacientes sufrieron un evento tromboembólico y dos pacientes ingresaron en UCI, siendo este último dato no objetivo debido a la falta de disponibilidad de camas UCI.

Como parte del seguimiento de la evolución clínica, se realizó serología de anticuerpos antiSARS-CoV-2 a los pacientes de la cohorte C+, de los cuáles sólo 2 pacientes (9,1%) desarrollaron inmunidad, uno a los 27 y otro a los 83 días. En 10 pacientes (45,5%) la PCR para SARS-CoV-2 fue positiva, sin ningún signo de aclaramiento viral hasta el fallecimiento o fin del seguimiento, estando este valor comprendido entre 10 y 197 días, con una mediana de 29 días.

No se encontró asociación con la dosis acumulada previa de rituximab ni con el tiempo trascurrido entre la dosis y la infección. La evolución de los pacientes según la clasificación de la OMS fue: 4 pacientes (categoría-1-asintomáticos), 1 (categoría-2), 0 (3), 5 (4), 4 (5), 3 (6), y 5 (éxitus-10) (Tabla 3). La tasa de mortalidad fue del 22,7% a los 60 días tras el resultado de la primera prueba positiva (corte del estudio). Ningún paciente había recibido vacunación frente a SARS-CoV-2 por no estar aún disponibles.

DISCUSIÓN

La tasa de mortalidad por COVID-19 en España se sitúa en torno a un 2% en el total de la población [8]. Nuestro estudio presenta una serie casos de pacientes con antecedentes de diferentes patologías por las que reciben rituximab y que sufren infección por SARS-CoV-2, produciéndose un 45,5% de casos de virus persistente sin ningún signo de aclaramiento viral (negativización de PCR) hasta final de seguimiento. Adicionalmente, en los casos más graves se produjo la fase inflamatoria de tormenta de citocinas durante el curso de la enfermedad y todos fallecieron por el deterioro respiratorio progresivo. Esto ha sido reportado en alguna serie de casos en pacientes inmunocomprometidos mayoritariamente con patologías hematológicas [9,10].

Rituximab se utiliza de forma muy eficaz en el tratamiento de diversas patologías diferentes, pero todas comparten una depleción de los linfocitos B y la supresión de su función, como puede ser la producción de anticuerpos anti-SARS-CoV-2, lo que parece ser la causa de infecciones graves y persistentes prolongadas.

Se debería tener en cuenta también que esta depleción de células B tras la terapia con rituximab puede conducir a una respuesta inmune humoral deficiente frente a la vacunación. Diversos estudios ya mostraron esta respuesta atenuada a la vacuna contra la influenza en pacientes tratados con rituximab [11].

Según la guía de recomendaciones de la *American College of Rheumatology* [12], los pacientes con un riesgo bajo o mitigable de COVID-19 deben recibir la vacunación frente a la COVID-19 alrededor de 4 semanas antes de su próximo ciclo de rituximab, y una vez completada la vacunación se debe retrasar el uso de rituximab de 2 a 4 semanas después de la segunda dosis de vacuna si la actividad de la enfermedad lo permite. Otra revisión centrada en reumatología recomienda evitar la vacunación después de rituximab hasta al menos 6 meses de su administración, y si la vacunación es inminente, se considera adecuado retrasar la administración de rituximab si no hay riesgo de progresión o brote de la enfermedad [13].

La vida media y los efectos prolongados de agotamiento de las células B del rituximab dificultan la obtención de una ventana óptima para la vacunación. Moor et al. [14] corroboran esta respuesta deficiente a las vacunas de ARNm frente a SARS-CoV-2.

Por tanto, se debería valorar, de forma individual el beneficio-riesgo de esta terapia y el uso de alternativas en función de la patología y el paciente, y siempre que sea posible, evitar la terapia con rituximab al menos mientras exista una elevada incidencia de infección por SARS-COV-2 y los pacientes no hayan logrado desarrollar inmunidad tras una vacunación efectiva, ya que los pacientes con función de células B disminuida y otros factores de riesgo, como terapias inmunosupresoras adiciona-

les, pueden tener un alto riesgo de curso fatal de la enfermedad después de la infección por SARS-CoV-2.

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CONFLICTO DE INTERESES

Los autores declaran no tener conflictos de intereses.

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Descripción clínica y epidemiológica de un brote grave de salmonelosis en una escuela infantil urbana

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RESUMEN

Objetivos. Se describe clínica y epidemiológicamente un brote de infección gastrointestinal por *Salmonella entérica* ser. (serotipo) Enteritidis, en una escuela infantil urbana, que conllevó elevada morbilidad e importante alarma social. La comunicación inmediata, así como el estudio adecuado del brote, en ambas vertientes, permitieron identificar el patógeno y establecer medidas de control en un plazo razonable de tiempo. Se discuten aspectos controvertidos como la indicación de antibioterapia o el momento de cierre del centro.

Material y métodos. Se recogió retrospectivamente información clínica, analítica y epidemiológica, y se revisó la metodología y resultados del estudio del brote.

Resultados. 57 niños (3-45 meses) de 92 asistentes al centro, fueron afectados y tuvieron confirmación microbiológica. Diarrea y fiebre fueron los principales síntomas. 74% acudieron al hospital, y 37% ingresaron, (estancia media 3,3 días). Fueron factores asociados al ingreso: deshidratación, elevación significativa de reactantes de fase aguda y coagulopatía. 12 recibieron cefotaxima parenteral. Se registraron 2 complicaciones: 1 bacteriemia y 1 reingreso. La sospecha inicial del origen del brote fueron los alimentos, pero el análisis de las muestras testigo fue negativo. 5 trabajadores fueron positivos (2 sintomáticos). Vigilancia Epidemiológica concluyó que el origen probable del brote fue un portador asintomático y la manipulación incorrecta de pañales. El centro permaneció cerrado 8 días. Se realizaron medidas de limpieza y desinfección, instrucción sobre cambio de pañales y seguimiento de portadores.

Conclusiones. La agrupación en tiempo y espacio de casos debe ser notificada inmediatamente para el control precoz del brote. Los niños pueden presentar formas graves de gastroenteritis por Salmonella.

Palabras clave: diarrea; brote Salmonella; epidemiológico.

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Clinical and epidemiologic description of a severe outbreak of Salmonellosis in an urban nursery school

ABSTRACT

Objectives. We describe clinically and epidemiologically an outbreak of gastrointestinal infection by *Salmonella enterica* ser. (serotype) Enteritidis in an urban infant school, which led to high morbidity and significant social alarm. The immediate communication, as well as the adequate study of the outbreak, in both aspects, allowed identifying the pathogen and establishing control measures in a reasonable period of time. Controversial aspects such as the indication of antibiotherapy or the moment of closing the center are discussed.

Methods. We retrospectively collected clinical, analytical and epidemiological information and we reviewed the methodology of the outbreak study and its results.

Results. A total of 57 children (3-45 months), were affected and had microbiological confirmation. Diarrhea and fever were the main symptoms. 74% went to the hospital and 37% were admitted (mean stay 3.3 days). Factors associated with admission were: dehydration, significant elevation of acute phase reactants and coagulopathy. Twelve patients received parenteral cefotaxime. There were 2 complications: 1 bacteremia and 1 readmission. The initial suspicion of the origin of the outbreak was food, but the analysis of the control samples was negative. Five workers were positive (2 symptomatic). Epidemiologic Surveillance concluded that the probable origin of the outbreak was an asymptomatic carrier and improper diapers handling. The center was closed for 8 days. Cleaning and disinfection measures were carried out, as well as instruction on diaper changing, and the carriers were followed

Conclusions. Clustering in time and space of cases should be reported immediately for early control of the outbreak. Children may present severe forms of Salmonella gastroenteritis.

Keywords: diarrhea; Salmonella outbreak; epidemiological.

INTRODUCCIÓN

Las zoonosis son enfermedades transmitidas entre animales y humanos mediante contacto directo, ambiental indirecto o alimentos [1]. Existen numerosos mecanismos de transmisión, y algunas comparten varios, complicando considerablemente el diagnóstico [2]. Los síntomas pueden ser leves y transitorios, o afectar gravemente diferentes órganos, conllevando elevada morbimortalidad, especialmente en grupos vulnerables, como los niños. En Estados Unidos, el número de intoxicaciones alimentarias alcanza los 48 millones de casos/ año, afectando la salmonelosis y la campilobacteriosis a 2 millones de personas / año [3,4]. Según el informe de 2017 de la Autoridad Europea de Seguridad Alimentaria (EFSA) y el Centro Europeo para la Prevención y el Control de las Enfermedades (ECDC), los microorganismos más comunes fueron *Campylobacter* spp. y *Salmonella* spp. [5]

Se estima que *Salmonella* spp, causa anualmente en el mundo, más de 90 millones de enfermedades asociadas a diarrea, relacionadas con alimentos el 85% de casos, con elevada mortalidad, y especialmente en menores de 4 años, infectados con serotipos *Salmonella enterica* ser. (serotipo) Enteritidis (S. Enteritidis) y *Salmonella enterica* ser. Typhimurium (S. Typhimurium) [4].

Además del impacto sanitario, conllevan importante repercusión económica: costes de hospitalización, absentismo laboral, cese de ventas y procedimientos judiciales [6].

En España, la Red Nacional de Vigilancia Epidemiológica (RENAVE) se ocupa de la recogida sistemática de la información epidemiológica, su análisis e interpretación y la difusión de resultados, con el fin de reducir la incidencia de enfermedades transmisibles [7]. Los casos se declaran según los criterios de clasificación (sospechoso, probable y confirmado) de los Protocolos de las Enfermedades de Declaración Obligatoria (EDO), aprobados por el Consejo Interterritorial del Sistema Nacional de Salud del Ministerio de Sanidad en 2013 [8]. Los informes anuales de RENAVE incluyen la salmonelosis dentro del grupo de *enfermedades transmitidas por alimentos y agua*, siendo en nuestro país, de nueva vigilancia, desde 2015 [7]. Previamente, su notificación al Servicio de Información Microbiológica (SIM) era voluntaria. La información previa a 2017 es incompleta y el análisis de la tendencia temporal limitado. Según este informe, en 2017 y 2018, 13 Comunidades Autónomas y las ciudades de Ceuta y Melilla notificaron 9.757 y 8872 casos respectivamente; con una tasa de incidencia global por 100.000 habitantes (TI) de 29,74 y 27,77 casos respectivamente, siendo la TI más elevada en el grupo de 1-4 años, en correlación con la literatura [4,7].

Los casos se relacionan mayoritariamente con el consumo de huevos y lácteos [9] y casi la mitad de los brotes notificados se producen en el ámbito familiar, seguido de la restauración [7]. Los animales de granjas suponen el principal reservorio, además de algunas mascotas. Estos microorganismos pueden atravesar la cadena alimentaria, desde los piensos hasta los hogares o los establecimientos [10]. La prevención y en su caso, el rápido diagnóstico y notificación del brote, son fundamentales para frenar la diseminación y atenuar sus consecuencias [7,10].

Salmonella es un género de bacilos gramnegativos, anaerobios facultativos, de la familia *Enterobacteriaceae*, con gran capacidad para adaptarse a las condiciones ambientales. Su nomenclatura es compleja, subdividiéndose en: especies, subespecies, subgéneros, grupos, subgrupos y serotipos (serovares) [11]. El género Salmonella se divide en dos especies: *Salmonella bongori* y *Salmonella enterica*, que contiene seis subespecies (I, II, IIIa, IIIb, IV y VI) y más de 2500 serotipos, con diferente patogenicidad [4,11,12]. Pueden causar complicaciones, como bacteriemia, meningitis y osteomielitis. Los serotipos Enteritidis y Typhimurium son los más importantes implicados en la transmisión de animales a humanos en la mayor parte del mundo [4].

Un aspecto muy controvertido en el manejo de infecciones gastrointestinales es la antibioterapia, y las resistencias a esta, un grave problema. El último informe del ECDC y la EFSA sobre resistencias en Europa, confirma el descenso de efectividad de los antibióticos frente a *Campylobacter* spp y *Salmonella* spp, especialmente de las fluoroquinolonas, y un 28,3 % de *Salmonella* spp multirresistente [13,14].

No se recomienda de rutina el tratamiento antibiótico en niños > 12 meses e inmunocompetentes en gastroenteritis (GEA) con síntomas leves o moderados; pero sí cuando existen factores de riesgo (niños < 6-12 meses e inmunocomprometidos) [14,15] o en formas graves. Son síntomas de gravedad: diarrea con > 9-10 deposiciones/ día, fiebre alta/persistente o necesidad de hospitalización. La presencia de sangre en heces no condiciona indicación de antibiótico [15]. Los riesgos de esta terapia son los posibles efectos secundarios, el estado de portador, y la infección posterior por *Clostridioides difficile* toxigénico, siendo además controvertido su utilidad en la recuperación clínica. En casos graves, el potencial de mejora y prevención de complicaciones parece superar los riesgos, aunque no se ha demostrado en ensayos aleatorizados controlados con placebo [15]. Las fluoroquinolonas serían de elección por su actividad contra patógenos entéricos gramnegativos, elevada concentración tisular e intracelular, y escasos efectos secundarios. Estos antibióticos pueden usarse también con seguridad en niños, durante cursos cortos, [16] sobre todo cuando existen contraindicaciones para otras opciones disponibles, como trimetroprim-sulfametoxazol (TMT/SMX), cefixima o azitromicina [15]. En casos graves, estaría indicado cefalosporinas de 3ª generación, durante 7 - 14 días en bacteriemia y hasta 4-6 semanas en meningitis [14].

En los glosarios epidemiológicos se define brote como: episodio en el cual 2 o más casos de la misma enfermedad tienen alguna relación entre sí, teniendo en cuenta el momento de inicio de los síntomas, el lugar donde ocurrieron o las características de los afectados [17]. La investigación de un brote infeccioso tiene como objetivos la identificación de las causas y la adopción de medidas de control, pero también conocer el comportamiento de la enfermedad y los factores de riesgo en la población. La tabla 1 recoge el proceso de investigación de un brote [18].

Tabla 1 Pasos en la investigación de un brote[a]

1. Confirmar la existencia del brote
2. Definir e identificar los casos
3. Revisión bibliográfica
4. Organizar el equipo de trabajo
5. Encuesta epidemiológica
6. Descripción epidemiológica del brote.
7. Plantear hipótesis
8. Testar la hipótesis mediante:
Estudios clínico-epidemiológicos
Estudios de laboratorio y/o ambientales
9. Interpretar los datos
10. Aplicar las medidas de prevención y control
11. Comunicar los hallazgos
12. Cierre del brote e informe final

[a]Modificada de Horcajada et al [18]

Tabla 2 Datos epidemiológicos

Datos	N (%)
Presencia de síntomas (N=92 niños y 30 trabajadores)	
Niños	57 (62)
Trabajadores	2 (6,6)
Estudio microbiológico heces positivo	
Niños (57)	43 (75,4)
Trabajadores (30)	5 (16,7)
Edad (meses)[a]	
7-12	12 (27,9)
12-24	15 (34,9)
24-45	16 (37,2)
Edad media	20,5
Sexo[a]	
Niña	18 (41,9)
Niño	25 (58,1)
Hábitat residencia[a]	
Urbano	43 (100)
Rural	0 (0)

[a]Datos referidos a los 43 niños incluidos en el análisis.

En octubre de 2020, una escuela infantil urbana, con 92 niños y 30 trabajadores, se vio afectada por un brote de *S.* Enteritidis, que motivó varios ingresos hospitalarios, y la activación del Servicio de Vigilancia Epidemiológica (SVE).

El objetivo de este artículo es analizar el manejo clínico y epidemiológico de este brote, discutir las controversias en el uso de antibioterapia en estos pacientes y recordar la importancia del factor tiempo en la investigación etiológica.

MATERIAL Y MÉTODOS

Se realizó un análisis descriptivo, retrospectivo y observacional, incluyéndose todos los niños del centro con resultado positivo para *Salmonella* spp. en heces. Fueron excluidos niños sintomáticos sin confirmación microbiológica.

Variables recogidas: edad, sexo, fecha de inicio y fin de síntomas, fecha y duración del ingreso, síntomas, pruebas complementarias, resultados microbiológicos y tratamientos. Se registró la existencia de patologías previas o factores de riesgo.

Para el diagnóstico microbiológico de enteropatógenos en heces se utilizaron paneles sindrómicos basados en PCR a tiempo real (BioFire® FilmArray gastrointestinal GI) que detecta 22 dianas: bacterianas (*Campylobacter* spp., *Clostridioides difficile, Salmonella* spp., *Shigella* spp./*E. coli* enteroinvasiva, *E.coli* diarreogénicos, *Vibrio* spp., *Vibrio cholerae, Yersinia enterocolitica, Plesiomonas shigelloides*); víricas (Adenovirus 40/41, Rotavirus, Norovirus GI/GII, Astrovirus y Sapovirus) y parasitarias (*Giardia intestinalis*, *Entamoeba histolytica*, *Cyclospora cayetanensis* y *Cryptosporidium* spp.). Para el cultivo se utilizó caldo selenito y agar Hektoen. La identificación se realizó mediante MALDI-TOF (Biotyper system-Bruker Daltonics). La sensibilidad antibiótica se determinó mediante microdilución (MicroScan®). Para la serotipificación se siguió el esquema de Kauffmann-White [19].

Se solicitaron datos de estudio, vigilancia y seguimiento del brote al SVE de la Comunidad. El proceso se inició con la solicitud de información sobre los menús consumidos en el período entre 48 horas antes del 1º caso hasta el último día de la semana anterior. Inspectores de la Unidad Territorial de la Agencia (UTA) recogieron las muestras testigo de alimentos, que fueron analizadas en el Laboratorio de Salud Pública, y se realizó un cribado mediante coprocultivo a los trabajadores.

Análisis estadístico. Las variables se analizaron con el programa SPSS 17.0. Para la descripción de la muestra se usaron la media, mediana y desviación estándar (DS). El contraste de hipótesis se realizó mediante el test t de Student, la prueba ANOVA para variables cuantitativas y la Chi cuadrado para variables categóricas. Un valor de $p < 0,05$ fue considerado significativo.

RESULTADOS

Al centro, que disponía de cocina propia, asistían 92 niños (edad: 3-45 meses), distribuidos en 8 aulas: < 12 meses (2

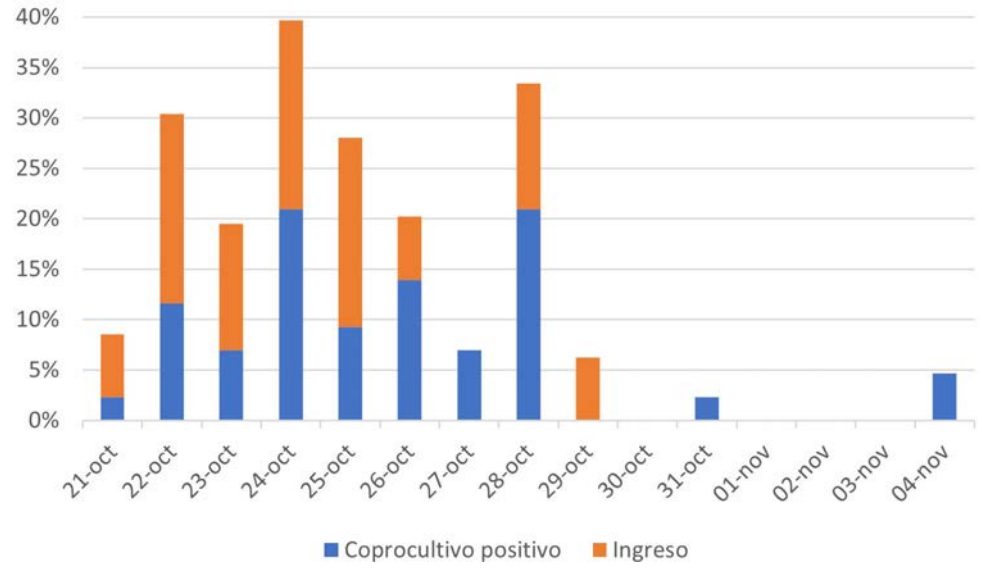

Figura 1 Evolución epidemiológica del brote.

El primer ingreso ocurrió el día 21 de octubre y el último el día 29. Los ingresos se concentraron especialmente los días 24 y 25 de octubre (8 niños). El mayor porcentaje de coprocultivos + corresponde a los días 24 y 28 de octubre (9 positivos).

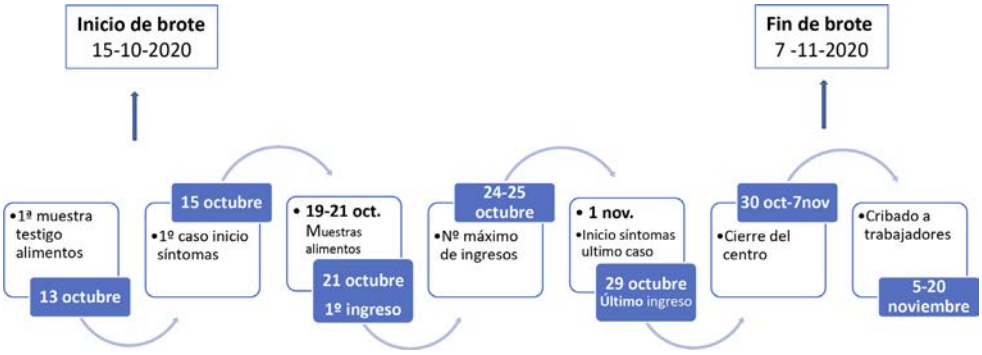

Figura 2 Línea temporal del brote.

El brote se desarrolló desde el punto de vista clínico, entre los días 15 de octubre (1º niño con síntomas) y el 1 de noviembre (inicio de síntomas del último de los casos); y desde el punto de vista epidemiológico entre los días 26 de octubre (inicio de estudio de menús) y 20 de noviembre (fin de cribado entre los trabajadores).

aulas), 12-24 meses (3 aulas) y > 24 meses (3 aulas) y 30 trabajadores. 3 niños presentaban patología: foramen ovale permeable, déficit de alfa-1-antitripsina y hemiparesia congénita.

Un total de 57 niños y 2 trabajadores presentaron síntomas compatibles con salmonelosis, detectándose *Salmonella* spp en heces de 43 alumnos y 5 profesionales. Los niños menores de 12 meses fueron los primeros en manifestar síntomas, agrupados en los primeros 6 días. Los mayores de 24 meses comenzaron con síntomas 8 días después del 1º.

La tabla 2 recoge los datos epidemiológicos y la tabla 3 datos clínicos. La tabla 4 muestra la correlación entre estos con el evento "ingreso" y la tabla 5 el nº de ingresos y estancia media, por grupo de edad.

Los antibióticos fueron mayoritariamente prescritos en hospitalizados (83,3%). La pauta predominante fue cefotaxima al ingreso (mantenido entre 1-3 días), y posteriormente amoxicilina (completando 7 días de tratamiento total, excepto en el caso que presentó bacteriemia, que fue tratado un total de 14 días). 2 pacientes tratados en atención primaria recibieron

Tabla 3 Datos clínicos de los niños sintomáticos.

Variables	Pacientes (n)	Porcentaje (%)
Síntomas		
Fiebre	35	81,4
Disminución ingesta	25	58,1
Vómitos	9	20,9
Diarrea	41	95,3
Sin productos patológicos	28	65,1
Con productos patológicos	13	30,2
Dolor abdominal	3	7
Atención médica		
Atención Primaria	10	23,2
Urgencias Hospital	32	74,4
Ambos	1	2,3
Pruebas complementarias		
Gasometría	20	46,5
Coagulación	19	44,2
Bioquímica	22	51,2
Hemograma	22	51,2
Virus en exudado faríngeo	20	46,5
Ingreso		
Si	16	37,2
No	27	62,8
Tratamiento recibido		
Rehidratación oral	14	32,5
Rehidratación intravenosa	14	32,5
Antibioterapia	12	28
Lactobacillus reuteri	8	18,6
Vitamina K	6	14

TMP/SMX (5 días) y amoxicilina-clavulánico (7 días) respectivamente.

La administración de vitamina K se realizó con el siguiente punto de corte: Tiempo de protrombina ≥15,6 segundos y/o tasa ≤ 62%.

La PCR en heces fue positiva en los 43 niños incluidos en el estudio y el cultivo en 41 de ellos. Se detectaron coinfecciones en 8 pacientes (1 con *Yersinia enterocolítica* y 7 *E.coli* enteropatógeno).

En el 97,7% de casos, *S.* Enteritidis fue sensible a amoxicilina/ampicilina o TMP/SMX, siendo resistente in vitro a amoxicilina/ampicilina en 1 caso, que fue sensible al resto de opciones (cefalosporinas y TMP/SMX).

Tabla 4 Variables epidemiológicas y analíticas en niños sin / con ingreso.

Variables	No ingreso	Ingreso	p
Sexo (N/%)			
Varones	10/23,3	8/18,6	0,405
Mujeres	17/39,5	8/18,6	
Edad (meses)	21,33 (9,28)	16,54 (9,74)	0,054
Grupos de edad (N/%)			
< 12 meses	4 /9,3	8 /18,6	
12-24 meses	12/27,9	3 / 7	0,084
> 24 meses	11/25,6	5/11,6	
Parámetros analíticos (media/DS o % de pacientes)			
PCT (ng/mL)	0,50 (0,24)	1,85 (1,78)	0,009
Elevación PCT	2 (33,3%)	12 (75%)	0,07
PCR (mg/dL)	4,88 (1,20)	5,24 (3,46)	0,784
Creatinina (mg/dL)	0,31 (0,26)	0,32 (0,12)	0,72
Urea (mg/dL)	18,17 (4,26)	18,57 (7,43)	0,93
Alteración función renal	0 (0)	3 (21,4%)	0,568
Alteración cifra leucocitos	0 (0)	5 (31,3%)	0,297
Tiempo tromboplastina (segundos)	13,52 (0,81)	15,72 (2,70)	0,07
Antibioterapia	2 (7,4%)	10 (62,5%)	<0,001

PCT= Procalcitonina. PCR= Proteína C reactiva.

Los resultados del estudio del brote objetivaron 5 trabajadores con coprocultivo positivo para *S.* Enteritidis; 2 sintomáticos en el momento del mismo; 3 trabajadores no realizaron cribado y en 22 fue negativo. Las muestras testigo de alimentos analizadas fueron negativas.

La calificación sanitaria del centro, por parte de los inspectores, fue "buena", pero se solicitó la realización de una limpieza y desinfección de las instalaciones. El centro se mantuvo cerrado una semana por orden de la Consejería de Educación para terminar la transmisión intracentro, y se solicitó una instrucción para realizar el cambio de pañales homogéneamente.

La Figura 1 muestra la curva epidemiológica y la Figura 2 la línea temporal con sus diferentes eventos.

La duración del brote desde el inicio de síntomas en el 1º niño hasta al menos dos periodos de incubación (6 días) sin casos nuevos fue de 3 semanas y 2 días.

DISCUSIÓN

Se describe un brote de GEA por *S.* Enteritidis, en un centro escolar urbano. 57 niños presentaron síntomas compatibles. El patógeno fue detectado en el 75,5% de muestras de heces de niños sintomáticos, y en 5 trabajadores (3 asintomáticos). Las muestras testigo de alimentos analizadas fueron ne-

Tabla 5 **Relación entre la edad, ingreso y días de estancia media.**

Grupo de edad	Ingresados (%)	Días (media ± desviación estándar)
<12 meses	8 (66,7%)	4,00 ± 2,27
12-24 meses	3 (20%)	1,50 ± 0,71
>24 meses	5 (31,3%)	3,00 ± 1,23

P>0,05

gativas. El origen se consideró un portador asintomático y la manipulación inadecuada de pañales.

La sintomatología fue común a la gastroenteritis por cualquier patógeno [20], no siendo la presencia de sangre en heces un signo predominante, ni motivo de ingreso, siendo más propio esto de infecciones por *Shigella* spp. y *Yersinia* spp. [4,15]. Contar con técnicas moleculares de diagnóstico sindrómico urgente para infecciones gastrointestinales permitió la rápida identificación del germen y la asociación precoz entre casos. En este brote, un 21% de casos estaban coinfectados también para otros gérmenes (1 *Y. enterocolitica*, y diversas cepas de *E. coli* diarregénico en 8 niños) detectados por PCR. Un 24,5 % de niños sintomáticos no tuvieron confirmación microbiológica, lo que no descarta el diagnóstico dada la coincidencia témporo-espacial con los casos. Un 38% de niños asistentes fueron asintomáticos. Otros estudios de brotes recogen datos similares, con cifras de asintomáticos más elevadas (81%) [20].

El elevado número de afectados e ingresos, probablemente obedece al rango de edad (media: 20,5 meses). Aunque no hubo diferencias significativas atribuibles a la edad en el evento "ingreso" en el global de la muestra, otros autores sí encuentran estas cuando el brote incluye niños mayores, siendo la tasa de ingresos y gravedad de estos muy superior en < 2 años [9,20].

Los síntomas y signos asociados al ingreso coincidieron con los descritos como indicadores de gravedad: diarrea con > 10 deposiciones/día, deshidratación, fiebre elevada y afectación general [21,22]. En > 50% de los niños sintomáticos se realizaron pruebas de laboratorio diferentes al estudio de heces en el entorno hospitalario y sin excepción en los ingresados. Esta tendencia en las unidades de urgencias frente a los servicios de atención primaria, facilitada por la mayor accesibilidad, parece justificada por la gravedad y edad de los afectados [15,22]. Los parámetros analíticos significativamente correlacionados con la hospitalización fueron: elevación de cifras de urea, creatinina y reactantes de fase aguda y coagulopatía (atribuible a la infección y al déficit de ingesta). El estudio microbiológico de heces no está indicado de rutina en las GEA, pero sí en los brotes, (especialmente en escuelas, guarderías y hospitales), para identificar el patógeno y establecer su origen [22,23]. Este brote fue rigurosamente estudiado a nivel microbiológico.

Existió cierta discrepancia entre profesionales, en cuanto a la prescripción de antibióticos en hospitalización. 10 pacientes ingresados los recibieron, y 2 en atención primaria. Este resultado es esperable y congruente con la mayor gravedad de los niños que ingresan. La indicación obedeció a la fiebre elevada y persistente, la corta edad (8 niños < 12 meses), importante elevación de reactantes de fase aguda y un caso de bacteriemia. Alguna guía restringe el criterio de edad a < 3 meses, para evitar complicaciones como la bacteriemia y focos extraintestinales. En base a esto, y dado que nuestros pacientes eran mayoritariamente sanos y >3 meses, probablemente se pautó antibioterapia en exceso [22]. Además de sus efectos secundarios, ésta puede alargar el tiempo de eliminación del patógeno en heces, y contribuir a la generación de resistencias, importante motivo de preocupación mundial [24,25]. Solo 1 niño presentó resistencia in vitro de *S.* Enteritidis a amoxicilina/ampicilina, siendo sensible al resto de opciones terapéuticas.

La elección empírica del antibiótico estuvo acorde con las recomendaciones, ya que la mayoría de ingresados recibieron una cefalosporina parenteral, y amoxicilina al alta [15,22].

Uno de los niños reingresó 20 días después del alta por reaparición de diarrea, siendo la detección en heces positiva para *C. difficile* toxigénico La interpretación de este resultado fue discutida, por la edad del paciente (7 meses), pero el ingreso y la antibioterapia parenteral previa con cefotaxima fueron considerados factores determinantes de la complicación [23,26]. Como parte del tratamiento, 8 de los niños, incluido el afectado, recibieron probióticos (Lactobacillus Reuteri) por su potencial beneficio en la prevención del *C. difficile* toxigénico en hospitalizados [27], no siendo efectivo en este caso.

No hemos encontrado en la literatura publicaciones sobre brotes por *Salmonella* en colegios de nuestro país. Nuestra Comunidad Autónoma se encontraba en 2017, según el último informe anual, entre aquellas con tasa de incidencia más baja, junto con Canarias, Madrid y Cataluña [7]. Existen casos documentados de brotes por *Salmonella* en relación con alimentos en adultos. En 2014 se comunicó un brote de gastroenteritis por *Salmonella* en Bizkaia, que afectó a 6 adultos, y tuvo su origen en chorizo casero de un mercado ambulante [28]. En 2016 otro similar afectó a 112 adultos de 7 Comunidades, asistentes a una concentración de motos en Valladolid, en relación con el consumo de bocadillos de carne de cerdo asada [29]. El serotipo responsable fue Typhimurium en ambos casos; sin embargo, se describe como más frecuente Enteritidis [7,30], como ocurrió en nuestro brote.

A partir del 2º caso detectado procedente del mismo centro, se realizó la notificación al SVE que coordina todo el proceso de estudio y se encarga de la comunicación a nivel nacional o internacional si procediera. La diligencia en el estudio es vital para frenar la extensión del brote y minimizar la carga de enfermedad. En nuestra Comunidad, los Agentes Coordinadores de Área o los Técnicos de Salud inician las diligencias de investigación sobre el terreno: encuestas, indicación de cuarentenas, estudio de contactos, etc. A continuación, y en función del patógeno implicado, se contactará con la Agencia de Seguridad Alimentaria y Sanidad Ambiental, que realizará

inspecciones (registros, actas, toma de muestras…). Al final se notifican los resultados a la entidad afectada.

La identificación del patógeno permite conocer el periodo de incubación (PI) y este prever la aparición de los casos durante el brote y determinar su cierre, considerado cuando hayan pasado 2 PI sin casos nuevos. Un informe final recoge el proceso de investigación y sus resultados. En este caso el PI se estima entre 6-72 horas [4,31]. Disponer de técnicas moleculares de diagnóstico sindrómico para el estudio de heces facilitó el diagnóstico microbiológico en muy breve plazo.

La hipótesis inicial, al compartir aula y comedor escolar los primeros niños afectados, fue la transmisión alimentaria, pero no pudo confirmarse al resultar todos los análisis de alimentos negativos. La positividad en 5 trabajadores que no comían en el centro, y de los cuales 3 fueron asintomáticos y la revisión de la manipulación de pañales, llevó a la conclusión final de que el origen del brote había sido un portador adulto y que un inadecuado manejo en los cambios de pañal provocó la extensión del mismo.

El hecho de que los primeros 8 niños sintomáticos fueran < 12 meses, habiendo además un periodo de 8 días hasta la aparición de síntomas en el primer niño > 2 años, parece congruente con los resultados de la investigación, al ser estos lactantes los que más cambios de pañal requieren y algunos > 2 años, incluso continentes.

A los trabajadores positivos se les hicieron recomendaciones higiénicas y se realizaron controles mediante coprocultivo hasta negativización, evitando la asistencia al trabajo. En cualquier caso, desde la puesta en marcha de los sistemas de autocontrol APPCC (Análisis de Peligros y Puntos de Control Crítico), la recomendación principal es el rigor en el ejercicio de las tareas de los manipuladores, ya que en ese caso nunca contaminarían con sus bacterias de origen fecal, aún siendo portadores asintomáticos [32].

Podríamos considerar que la duración total de este brote, habiendo conocido el patógeno responsable en las primeras horas de los 2 primeros casos, fue larga, pero el proceso de identificación de portadores entre 30 trabajadores, y la identificación del factor ambiental asociado conllevó su tiempo. Quizá como reflexión final, se podría plantear si el momento del cierre del centro (día 9 tras el primer caso) fue el adecuado o podría haberse realizado antes. Las modernas técnicas microbiológicas deben conllevar también medidas rápidas epidemiológicas.

En resumen, ante un brote, la rápida identificación del patógeno es vital para conocer los riesgos de la población, estimar la morbilidad esperada, valorar tratamientos necesarios, establecer el periodo de incubación y buscar las fuentes más probables originarias del mismo.

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CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

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Real-life experience of hepatitis C treatment in a Spanish prison

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ABSTRACT

Background. Hepatitis C virus (HCV) infection is a major public health problem that causes multiple comorbidities. People in prisons who inject intravenous drugs are at increased risk of HCV infection, and HCV infection is 15-fold more prevalent among prisoners compared with the community. The objective of this study was to analyse the clinical and epidemiological characteristics of residents of a Spanish prison with HCV infection who received antiviral treatment.

Material and methods. An observational, descriptive and retrospective study was performed. All patients with HCV infection diagnosed or followed up in an Infectious Diseases attached to a penitentiary were included in this study.

Results. Of 81 patients analysed, sixty-nine (83.1%) patients were male. The mean age was 50.1 (SD8.8) years, and 70% of the inmates had a history of injection drug use. Coinfection with HIV was detected in 30%. In up to 25% of the sample, there were data on chronic liver disease in the degree of liver cirrhosis. The diagnosis of HCV infection had been made more than 15 years earlier in 28% of those studied. Decompensations from liver disease, hepatocellular carcinoma, or hospital admissions were exceptional. Most of the inmates with HCV accepted treatment, and approximately 94% of the patients who completed treatment achieved a sustained virological response without interactions or complications of interest.

Conclusions. The availability of direct-acting antivirals and their exceptional side effects constitute an opportunity to reduce the burden of HCV infection in Spain, particularly in these high-risk populations.

Keywords: Hepatitis C; liver cirrhosis; disease eradication; treatment; prisoners

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Tratamiento de la hepatitis C en un centro penitenciario Español

ABSTRACT

Introducción. La infección por el virus de la hepatitis C (VHC) es un importante problema de salud pública con una gran morbimortalidad. El consumo de drogas inyectables es la principal vía de transmisión, siendo la infección por VHC 15 veces más prevalente en las cárceles españolas respecto a la comunidad. El objetivo de este estudio fue analizar las características clínico-epidemiológicas de los residentes de un centro penitenciario con VHC que recibieron tratamiento.

Material y métodos. Estudio observacional, descriptivo y retrospectivo. Se incluyeron en este estudio todos los pacientes con infección por VHC diagnosticados o seguidos en una Unidad de Enfermedades Infecciosas adscrito a un centro penitenciario.

Resultados. De 81 pacientes analizados, sesenta y nueve (83,1%) pacientes eran varones. La edad media fue de 50,1 (DE 8,8) años y el 70% de los internos tenía antecedentes de consumo de drogas inyectables. Se detectó coinfección por VIH en 30%. En un 25% presentaban enfermedad hepática en grado de cirrosis. En el 28% de los internos el diagnóstico de VHC se había realizado hacia más de 15 años. Las descompensaciones por enfermedad hepática, carcinoma hepatocelular o ingreso hospitalario fueron excepcionales. El 94% de los pacientes que completaron el tratamiento lograron una respuesta virológica sostenida sin interacciones ni complicaciones de interés.

Conclusiones. La disponibilidad de antivirales de acción directa y sus excepcionales efectos secundarios constituyen una oportunidad para reducir la carga de infección por VHC en España, especialmente en estas poblaciones de alto riesgo.

Palabras clave: Hepatitis C; cirrosis hepática; erradicación de enfermedades; tratamiento; prisioneros.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health concern globally with an estimated 71 million people infected worldwide [1]. When untreated, HCV can lead to cirrhosis, hepatocellular carcinoma and death [2]. Unsafe injecting drug use (IDU) is the main route of HCV transmission in developed countries with an estimated 20 million people who inject drugs infected [3].

In Spain, 1.6–2.6% of the population is infected with HCV, and IDU is the most common mode of HCV transmission [4,5]. People who inject drugs are overrepresented in prison populations and globally represent 20–55% of persons imprisoned. These individuals have higher levels of HCV infection than the general population [4,5]. HCV is 15-fold more prevalent among persons in Spain's prisons than in the community, and the HCV seroprevalence in Spanish prisons was 22.7% according to a survey performed in 2011 [6-8]. Therefore, prisons are HCV infection reservoirs.

This collective had some specific problems of the prison health care system, including excessive delays for specialized consultations, poor communication between specialists from reference hospitals and prisons doctors and health providers, missed appointments and logistical difficulties for inmate transfer to hospitals.

The Spanish Health Ministry Strategic Plan for hepatitis C infection highlighted intervention in prisons as a priority action. However, there are important barriers associated with specialized care provision to the penitentiary population [9,10].

With recent advances in treatment regimens, HCV is now a curable and preventable disease, and prisons provide an ideal opportunity to engage this hard-to-reach population [11,12].

Several recent studies have shown that treating prisoners in Spanish prisons with HCV using current direct-acting antiviral agents (DAAs) can be successful [13] and has a similar effectiveness to that observed in the nonincarcerated population [13]. Therefore, optimizing HCV treatment in prison inmates represents an opportunity to improve the health of these patients and has great epidemiological importance given the ability of this group to transmit the infection.

The objective of this study was to analyse the main clinical-epidemiological characteristics of residents of a Spanish prison with HCV infection who received antiviral treatment.

MATERIAL AND METHODS

Study design and participant selection. A retrospective, longitudinal, descriptive study was designed to review all patients diagnosed with HCV infection residing in Topas' Penitentiary Centre and assisted in Complejo Asistencial Universitario de Salamanca (CAUSA) between January 2015 and December 2021.

CAUSA is a tertiary care hospital. The internal medicine service of this hospital has approximately 130 beds allocated. The Infectious Diseases Unit attached to this service is organized based on general and monographic external consultations, serves as an area of hospitalization, and is a medical liaison with the penitentiary centre of the province.

Topas' Penitentiary Centre was opened in 1995 in the municipality of Topas in the province of Salamanca under the direction of the Ministry of the Interior, a department of the Government of Spain responsible for public security. The facility has the capacity to house 2,000 inmates.

Data collection. The clinical and epidemiological data were collected after a review of the medical records. All analysed data were anonymized. It was not necessary to contact any of the patients to perform this study. A review was performed according to the clinical protocol that included age, sex, nationality, province of residence, personal history of injecting drug use, type of sexual intercourse, transfusion of blood products, year of diagnosis, year of first evaluation in CAUSA, HCV genotype, stage of fibrosis by live elastography, MELD and CHILD index score, human immunodeficiency virus (HIV) coinfection, presence of liver disease and complications/hospital admissions, date of initiation of antiviral treatment, duration of treatment, medical follow-up, sustained virological response and reinfections.

Inclusion and exclusion Criteria: All patients over 18 years of age who were residents of Topas Prison and had a diagnosis of active HCV infection were included. Patients who did not meet these criteria or had missing data were excluded.

Definitions

MELD: The MELD (Model for End-stage Liver Disease) index is an objective and easily reproducible prognostic staging of mortality based on three simple analytical variables: bilirubin, serum creatinine, and the international normalized ratio of prothrombin time (INR). Various studies have shown that the MELD index estimates the mortality risk of patients on the transplant waiting list better than other prognostic indices [3]. Patients with a MELD greater than 18 points have a high risk of mortality and a median survival of less than 3 months [11].

CHILD: The Child–Pugh scale is a staging system used to assess the prognosis of chronic liver disease, mainly cirrhosis, and the need for liver transplantation. Each criterion is rated from 1-3, with a score of 3 indicating the most serious damage [2]. All the patients analysed had 5-6 points on the Child–Pugh scale, corresponding to class A, with a 2-year survival of 85% [14].

Portal Hypertension: Portal hypertension syndrome was noted if the patient had symptoms or signs usually associated with PH (including variceal haemorrhage, portal hypertensive gastropathy, ascites, and spontaneous bacterial peritonitis) and radiological signs on ultrasonography or tomography (such as ascites, splenomegaly, portal flow mean velocity < 12 cm/second, inversion of flow in the portal vein, portosystemic collaterals of a patent periumbilical vein or spleen-renal collaterals, dilated left and short gastric veins, portal vein diameter

0.13 mm, decreased or no respiratory variation in the diameter of the splenic and superior mesenteric veins, and portal, splenic, or superior mesenteric vein thrombosis). We also considered PH if liver stiffness was > 13.6 kPa as measured by FibroScan®.

Liver elastography: Ultrasound-based elastography is primarily used as an alternative to liver biopsy for the assessment of hepatic fibrosis. It is a type of ultrasound imaging that directs painless low-frequency vibrations into the liver to measure how quickly these vibrations move through it. A computer uses this information to create a visual map showing the stiffness of the liver. In patients with chronic hepatitis C, researchers [15] showed that liver stiffness measurements ranged from 2.4 to 75 kPa with a median value of 7.4 kPa. Based on the stiffness measurement distribution according to fibrosis stage and receiver operating characteristic (ROC) curves, we found that the cut-off value for cirrhosis was 12.5 kPa in patients with HCV infection.

Data analysis. To perform statistical analyses, descriptive analysis was conducted for each individual variable. For categorical variables, the results are expressed as absolute values (n), proportions (n/N), or percentages (%). For quantitative variables, the results are expressed as the mean and standard deviation (SD), median and interquartile range (minimum value–maximum value). The SPSS 26.0 (SPSS, IBM, Armonk, NY) statistical package was used.

Ethics statement. The study protocol (PI 2021 12 919) was approved by the Clinical Research Ethics Committee of Complejo Asistencial Universitario de Salamanca (Salamanca, Spain). All data analysed were anonymized. Exemption of informed consent was obtained from the University Assistance Complex of Salamanca Ethics Committee due to the retrospective nature of the study and the anonymized patient data. The procedures described here were performed in accordance with the ethical standards described in the revised Declaration of Helsinki in 2013.

RESULTS

Between 2015 and 2021, a total of 83 residents of the Topas Penitentiary Centre were candidates to receive antiviral treatment against HVC infection. Two patients finally refused the treatment. The main data of the 81 patients studied are shown in Table 1.

The mean age of the patients was 50.1 (SD 8.8) years, and 69 (83.1%) were male. Grouped by age, 9.6% were between 24 and 40 years old, 33.7% were between 41 and 50 years old, 45.8% were between 51 and 60 years old, and 10.9% were over 61 years old. Of the 83 patients studied, 57 (68.7%) were or had been injection drug users. Thirty-one percent of the patients were unaware of the method of transmission.

Sixteen (19.3%) of the patients had been diagnosed with HCV infection in the last 5 years. In 27.7% of the cases, the diagnosis had been made more than 15 years ago. Regarding complications, only one patient had required hospital admission due to oedema ascitic syndrome during the year prior to this study. None of the patients presented evidence of hepatic encephalopathy, upper gastrointestinal bleeding or hepatocellular carcinoma.

Table 1 Main epidemiological and clinical of prisoners with chronic hepatitis C virus infection

Variables	N= 83
Male gender (n, %)	69 (83.1)
Age, years (mean [SD])	50.1 (8.8)
Nationality (n, %)	
Spain	69 (83.1)
Other Western European country	2 (2.4)
Eastern Europe	4 (4.8)
Asia	2 (2.4)
Africa	3 (3.6)
Unknown	3 (3.6)
Comorbidities (n, %)	
HIV coinfection	25 (30.1)
Injecting drug users	57 (68.7)
Established liver disease (hepatic cirrhosis)	33 (39.8)
Stage F3	14 (16.9)
Stage F4	19 (22.9)
Portal hypertension	5 (6)
Hepatocarcinoma	0 (0)
Time from diagnosis to treatment (n, %)	
≤ 5 years	16 (19.3)
5-10 years	16 (19.3)
11-15 years	27 (32.5)
> 15 years	23 (27.7)
Unknown	1 (1.2)
Naïve patients (n, %)	64 (77.1)
Refusal of treatment (n, %)	2 (2.4)
Relevant complications of treatment (n, %)	0 (0)
Sustained virologic response (n, %)	76 (93.8)

Regarding characteristic data, two patients had been diagnosed with non-Hodgkin lymphoma when HCV infection was known; 7% were diabetic, and 20.5% had psychiatric illnesses, such as personality disorders, schizophrenia or bipolar disorder. In 25 (30.1%) patients, HCV infection coexisted with HIV infection.

The main analytical data related to HCV are shown in Table 2. Of the patients studied, 37% had mild hepatic cytolysis in the first follow-up test with a transaminase value less than 1.5 times the upper limit of normality without associated co-

Table 2 Main characteristics of HVC and liver disease in prisoners

Variables	N= 83
Child-Pugh (n, %)	
Child A	82 (98.8)
Child B	1 (1.2)
Child C	0 (0)
MELD (n, %)	
≤ 9	76 (91.6)
10-19	7 (8.4)
≥ 20	0 (0)
Genotype of HCV (n, %)	
1a	39 (47)
1b	5 (6.1)
2	2 (2.4)
3	16 (19.3)
4	13 (14.6)
1a/3a	2 (2.4)
Unknown	6 (7.2)
Fibrosis stages (sound bases electrography) (n, %)	
Stage F0	27 (32.5)
Stage F1	6 (7.2)
Stage F2	16 (19.3)
Stage F3	14 (18.9)
Stage F4	19 (22.9)
Unknown	1 (1.2)

MELD: Model for End-stage Liver Disease

agulopathy. Seven percent of inmates had mild thrombocytopenia with platelet counts greater than 100,000/µL.

A total of 33 patients (39.7%) with HCV infection had fibrosis score of F0 to F1 in the last liver elastography performed. Approximately 23% of the patients analysed had grade 4 fibrosis, indicating maximal fibrosis or cirrhosis. None of the patients analysed had a MELD index ≥ 19, so no case was registered in the pre-liver transplant programme.

Regarding the treatment, 17 (20.5%) patients had previously received interferon and ribavirin without achieving a sustained viral response. In addition, three patients had previously received treatment with interferon and ribavirin and a combination of direct-acting antivirals in other health centres. None of the patients had completed therapy, and the three patients maintained a detectable viral load.

The number of patients treated per year is shown in Figure 1. The combination of sofosbuvir and daclatasvir was used in patients with genotype 3; sofosbuvir and ledipasvir in genotypes 1 and 4; sofosbuvir and velpatasvir in genotypes 1, 3 and 4; and glecaprevir and pibrentasvir in genotypes 1-4. The prescribed direct-acting antivirals for HCV infection are detailed in Figure 1. Inmates' chronic medication was recorded prior to antiviral initiation to avoid drug interactions, especially with antiretroviral therapy against HIV. The duration of treatment varied between 8 and 24 weeks. Three patients were lost during the posttreatment follow-up due to transfer to other prisons, and two patients finally declined to start antiviral treatment. In total, 76 patients achieved a sustained viral response, representing 93.8% of those treated. The patients who remained in the province received follow-up by the Infectious Diseases Unit for at least six months without registering complications or notable interactions.

DISCUSSION

Our work, which involved greater than 80 inmates with multiple pathologies, coinfection with HIV and drug use who receive treatment with direct-acting antivirals against HCV, shows sustained viral response rates in greater than 93% of patients with few side effects.

The number of men in Spanish prisons exceeds that of women with a 12:1 ratio according to data from the Spanish Ministry of the Interior. The majority (83%) of the inmates studied were male, so the prevalence based on gender cannot be estimated without bias. It has been described in prevalence studies that HCV infection is more frequent in men between 50 and 59 years of age followed by the age group between 60 and 69 years [4]. In our study, half of the patients were between 51 and 60 years old.

On the other hand, greater than 80% of the inmates studied had Spanish nationality, so it is not possible to assess the prevalence of HCV infection or compare the genotypes of the virus with those of the foreign population.

Approximately one-third of the inmates with HCV infection studied had coinfection with HIV. This prevalence is slightly higher than that reported in other studies (approximately 26%) [16]. This subgroup had a mean age of 52 years (SD 8.8). The prevalence of HIV infection in prisoners in Spain is 10.8%. Those infected are usually injection drug users and over 40 years of age, and 85% are coinfected with HCV [16]. According to data from GeSIDA [17], the prevalence of HIV/HCV coinfection in Spain in 2018 was 3.7%, which represents a reduction of 83.3% compared to that observed in 2015 [18]. The increase in exposure to direct-acting antivirals against HCV was the cause of this drastic decline.

In parallel, approximately 70% of the analysed sample had a history of parenteral drug use. Injection drug users are a high-risk HCV infection group with a prevalence of up to 90% in Irish prisons [6,12], constituting the leading cause of infection in young adults. Other transmission methods, such as vertical transmission, transmission through blood products, sexual transmission or transmission due to piercing or tattoos, could not be estimated in our study due to the lack of data in

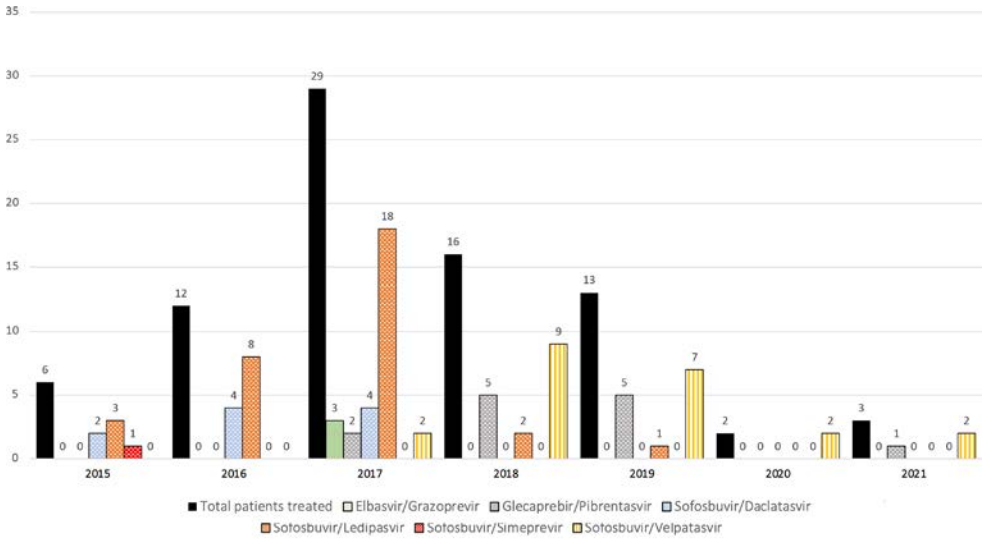

Figure 1 | **Direct-acting antiviral HCV prescribed to inmates of a penitentiary centre**

the medical records and the retrospective nature of this study.

In Spain, the most prevalent HCV genotype is genotype 1 with a predominance of 1a and 1b, which also coincides with the data obtained in our study [4]. In the group of foreigners, 62% also had genotype 1, 31% had genotype 3, and 8% had genotype 4.

Liver cirrhosis due to HCV is the main cause of chronic liver disease. Chronic inflammation of the liver can lead to liver fibrosis and eventually the development of cirrhosis. Approximately 25% of the patients analysed had grade 4 fibrosis estimated with non-invasive tests, although only a small percentage of patients presented decompensations or required hospital admission due to liver disease. No case of hepatocellular carcinoma was recorded.

Of note, 28% of those studied were infected with HCV for more than 15 years. It has been estimated that the risk of developing cirrhosis after 20 years of HCV infection without treatment ranges between 14 and 45%. In addition, once cirrhosis is established, the probability of developing decompensation or primary liver cancer is 20% at 5 years [19].

The efficacy of direct-acting antivirals for the treatment of HCV infection has been demonstrated in clinical trials, including in coinfected patients [19]. In our study, approximately 94% of treated inmates achieved sustained virological response. Spain started a pilot program to detect and treat HCV in prisons in 2016. The results of this study showed that a sustained viral response was achieved in greater than 95% of treated patients [7], and this result is consistent with our data. Toxicity and therapeutic abandonment were exceptional [20]. The COVID-19 pandemic is one of the main factors that explains the decrease in the number of cases treated in recent years.

Inmates in prisons have complex physical and psychological needs, which are not addressed by the primary health system. Different studies have shown that prisoners have better access to primary care and lower mortality when serving time in a prison compared to when they are released back to the community as a result of access to good nutrition, exercise, and a structured routine and the lack of stressors experienced [12]. Time in prison is an opportunity to treat hepatitis C. At the community level, these studies can make a great contribution to the country in controlling HCV infection.

The strategic plan of the Spanish Ministry of Health for HCV infection emphasized intervention in prisons as a key factor to address HCV infection. However, there are specific problems related to health care in prisons, including excessive delays in specialized consultations, poor communication between prisons and medical centres, a lack of providers and logistical difficulties in managing the transfer of inmates to hospitals [10].

In this sense, telemedicine offers a unique opportunity to eliminate geographic barriers, improve equity of access to specialized medical care, and guarantee continuity of care between hospitals and prisons.

Our study has methodological limitations derived from its retrospective nature and its inclusion of a small sample of inmates in a Spanish prison. The difficulty of accessing these patients and the interruption of follow-ups due to prison changes are other limitations of this work.

CONCLUSIONS

The wide acceptance and therapeutic adherence to antiviral treatment against HCV infection in a sample of inmates in a penitentiary centre with a high sustained virological re-

sponse rate and exceptional side effects is the main finding of our study. Antiviral treatment of patients with HCV infection is a priority, and it can be effectively provided in Spanish prisons with results equal to or better than those described in the community. Direct-acting antivirals have been shown to be safe and effective, even in coinfected patients. It is necessary to implement educational programs that highlight the benefits of these treatments in communities with limited access to the health system that are governed by the acceptance and reduction of stigma. Telemedicine has been employed in this environment to facilitate inmates' access to specialized care and as a comprehensive public health strategy.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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Bezlotoxumab in the treatment of *Clostridioides difficile* infections: a real-life experience

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ABSTRACT

Background. Following the approval of bezlotoxumab in 2017, studies evaluating its effectiveness in prevention of *Clostridioides difficile* infection under "real-life" conditions are scarce.

Material and methods. We conducted a retrospective study developed in a large tertiary care hospital describing the use and outcomes of patients with *Clostridioides difficile* infection (CDI) treated with bezlotoxumab.

Results. A total of 16 patients were include, all of whom had an episode of CDI with high probability of recurrence and 14 of them had some kind of immunosuppression. Bezlotoxumab was effective in the prevention of CDI recurrence in 11 of the 14 cases in which follow up was possible, without significant side effects.

Conclusions. Bezlotoxumab was well tolerated and the incidence of recurrent CDI in a high-risk population for recurrence was only 21.4%.

Keywords: *Clostridioides difficile*, bezlotoxumab, treatment, recurrence

Bezlotoxumab para el tratamiento de las infecciones por *Clostridioides difficile*: experiencia en vida real

RESUMEN

Antecedentes. Tras la aprobación de bezlotoxumab en 2017, son escasos los estudios que evalúan su eficacia en la prevención de la infección por *Clostridioides difficile* en condiciones de vida real.

Material y métodos. Realizamos un estudio retrospectivo desarrollado en un hospital terciario describiendo el uso y los resultados de los pacientes con infección por *Clostridioides difficile* (ICD) tratados con bezlotoxumab.

Resultados. Se incluyeron un total de 16 pacientes, todos ellos con un episodio de ICD con alto riesgo de recurrencia y 14 de ellos con algún tipo de inmunosupresión. El bezlotoxumab fue eficaz en la prevención de la recurrencia de la ICD en 11 de los 14 casos en los que fue posible el seguimiento, sin efectos secundarios significativos.

Conclusiones. El bezlotoxumab fue bien tolerado. La incidencia de ICD recurrente en una población de alto riesgo de recurrencia, fue sólo del 21,4%.

Keywords: *Clostridioides difficile*, bezlotoxumab, tratamiento, recurrence

INTRODUCTION

Clostridioides difficile infection (CDI) is an important cause of morbidity and mortality in hospitalized patients and it is an increasingly frequent cause of community onset diarrhea [1].

CDI has a high rate of recurrence [2], especially in patients with risk factors for recurrence such as: advanced age, prior CDI episodes, need to continue receiving antibiotics, immunosuppression (solid organ transplant, hematologic malignancy, neoplasia), infection by a hypervirulent strain (027 ribotype), concomitant Inflammatory Bowel Disease (IBD) or having a low toxin B Ct value in PCR test [3].

Recurrence rates for patients with previous recurrent episodes of CDI reach 45% when treated with metronidazole or vancomycin [4]. Moreover, in patients with multiple recurrences, the risk of further recurrences approaches 75% [5,6]. Recurrences increase days of hospital stay, readmissions and cost [7,8]. Fidaxomicin has been shown to reduce the rate of CDI to about 10% in first episodes [9] but it is not always available, since in Spain it is financed by the Ministry of Health for second recurrences.

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Fecal microbiota transplant emerged as an effective treatment for CDI and its recurring episodes, but it is not available everywhere, it should be given with caution in some immunosuppressed patients [10-15], and it is still considered an experimental procedure after a first episode of CDI.

Bezlotoxumab is a monoclonal antibody against toxin B of *C. difficile*, that is proven to be effective in decreasing the incidence of rCDI (recurrent CDI) in randomized clinical trials [16,17]. Experience in real-life situations in the post-marketing of bezlotoxumab are still scarce [18] and we are not aware of any particular cases published in Spain.

We report our experience in a Spanish tertiary hospital with the use of bezlotoxumab in the first 16 patients at high risk for CDI recurrence.

MATERIAL AND METHODS

Study design. This was a retrospective study, carried out in a large tertiary care hospital with 1,300 beds, in the 13-month period from the date of marketing bezlotoxumab in Spain (August 2018 – September 2019). We included in this study all patients who received Bezlotoxumab during that period, in our institution.

Patients were selected for bezlotoxumab if they fulfilled the indications for the financing of bezlotoxumab in Spain [20] and, in addition, had 3 or more risk factors for rCDI: age > 65 years, prior CDI episode, incapability to stop antibiotics during the CDI episode, immunosuppression (solid organ transplant, hematologic malignancy, neoplasia), infection by a hypervirulent strain such as 027 ribotype, concomitant Inflammatory Bowel Disease or with low toxin B Ct values. All the patients had a life expectancy of more than three months.

Medical records for all patients receiving bezlotoxumab were reviewed. Data collected included patient demographics, underlying conditions, number of former rCDI episodes, severity of CDI episode, treatment used for previous CDI episodes, antimicrobial therapy administered concomitant to bezlotoxumab, reasons for its use, dosages, adverse events and outcomes (need for ICU admission, need for surgery due to the CDI episode, recurrence, mortality, and CDI-associated mortality).

The laboratory diagnosis of *C. difficile* was performed according to our own practice [21]. Rapid tests were performed on all samples. The rapid detection test consisted of a two-step diagnostic algorithm based on a first immunochromatographic antigen detection of glutamate deshidrogenase (GDH) and toxins A/B simultaneously (CDiff Quik-Chek Complete assay, TechLab, Blacksburg, VA) and secondly, all samples with any of the previous tests positive, were tested by a real-time PCR of the B toxin gene (Xpert™C. difficile Assay, GeneXpert, Cepheid, Sunnyvale, CA). Furthermore, all samples were also tested by toxigenic culture (TC**)**.

Definitions. A CDI episode was defined as the presence of a positive result for toxigenic *C. difficile* testing (having found the toxin directly or indirectly) and the presence of diarrhea (3 unformed stools in 24 h) without other apparent cause.

Severity of CDI episodes was defined according to the European Society of Clinical Microbiology and Infectious Disease (ESCMID) guidelines [22].

CDI-associated mortality was defined as death, not clearly attributable to other causes, occurring within 10 days of the CDI diagnosis.

Recurrent CDI (r-CDI) was defined as CDI symptoms and positive stool sample that occurred in the first 10 to 90 days after recovery of a previous CDI episode.

The follow-up period of the patients was 90 days or until their death.

Ethical issues. Being a systematic clinical intervention, the local ethics committees approved the study, without requiring informed consent (MICRO.HGUGM.2019-021).

RESULTS

In the 13-month period from the date of marketing bezlotoxumab in Spain, 16 patients fulfilled our criteria for bezlotoxumab selection and accepted this modality of treatment in our center.

Demographic and baseline characteristics of the study population were shown in Table 1. Patients' age ranged from 54-84 years [median 69.5 years; SD 20 years] and 10 (62.5%) were female. Overall, 14 (87.5%) had some kind of immunosuppression, 3 (18.8%) were solid organ transplant recipients, 6 (37.5%) patients had a cancer and 2 (12.5%) had cirrhosis. Overall, 8 (50%) of the patients received some kind of immunosuppressive therapy.

At presentation time, the disease was mild in 7 cases (43.8%), severe in 3 (18.8%) and severe-complicated in 6 (37.5%). Of the 16 patients, 9 (56.3%) had a history of prior CDI episodes, 15 episodes/recurrences in total treated with either metronidazole (1), vancomycin (7), extended duration vancomycin (2) or fidaxomicin (5).

During the episode treated with bezlotoxumab, 14 of the 16 patients received 10 days of vancomycin as concomitant treatment and two received 10 days of fidaxomicin due to a history of vancomycin allergy. We used standard dosages of bezlotoxumab (10mg/kg) in all patients. Bezlotoxumab tolerability was good and no side effects were detected.

Two patients died due to other causes, so it was not possible to demonstrate any CDI cure. Of the remaining 14 patients, 11 did not recur during a 3-month follow up period. This resulted in a recurrence rate of 21.4% (3/14).

In the subgroup of patients with any previous recurrence, the recurrence rate was 25% (2/8, because one died). These patients had had 2 and 4 respectively recurrences previously.

Table 1 Description of patients and their results

Nº of patients	Age	Gender	Underlying conditions	Risk factors present	Immunosuppresive therapy	Episodes or Recurrences	Severity	Previous treatment	Concomitant treatment	Outcome
1	69	F	Kidney transplant	4	Tacrolimus, Everolimus, Prednisone	1	Severe	VAN		Curation
2	66	F	Micobacterium avium lung infection	3	no	4	Mild	VAN and FID	VAN	CD Recurrence
3	57	F	Oropharyngeal cancer, intestinal perforation (colostomy)	3	Radiotherapy & Chemotherapy	1	Severe-complic.	VAN	VAN	Curation
4	80	F	Leukemia, myelodyspl. syndrome, RA, knee prosthesis infection	3	Azatioprine	3	Mild	MET and FID	FID (alergy to VAN)	Curation
5	84	M	Pneumonia, dysphagia	4	no	1	Mild	VAN	VAN	27/2 exitus
6	70	F	Kidney transplant	3	Tacrolimus, mycophenolate	0	Severe	-	VAN	Curation
7	57	M	Child C Cirrhosis	3	no	0	Severe-complic.	-	VAN	Curation
8	82	M	Lung cancer	3	Nivolumab	0	Mild	-	VAN	Curation
9	59	F	Ulcerative Colitis	3	AZA, 5ASA; Adalimumab, INF, GC	0	Severe	-	VAN	Curation
10	78	M	Child C Cirrhosis	3	no	0	Severe-complic.	-	VAN	Curation
11	80	M	Sigma cancer, cardiopathy	4	no	2	Mild	VAN	VAN	CD Recurrence
12	69	F	HIV	3	Other, HIV treatment	1	Severe-complic.	VAN	VAN	Curation
13	63	M	Pancreatic cancer	3	Chemotherapy	0	Mild	-	VAN	22/07 exitus
14	76	F	Pancreatic cancer	5	Chemotherapy	1	Severe-complic.	VAN	VAN	Curation
15	81	F	Renal insufficiency, cardiopathy	3	no	1	Mild	FID	FID (alergy to VAN)	Curation
16	54	F	Renal insufficiency, liver transplant	3	Tacrolimus	0	Severe-complic.	-	VAN	CD Recurrence

Risk factors for rCDI: age >65 years, prior CD episode, antibiotic use during standard of care, immunosuppressed (solid organ transplant, hematologic disease, neoplasia), patients infected by a hypervirulent strain such as 027 ribotype, concomitant Inflammatory Bowel Disease or with low toxin B Ct values.
Gender: (F: Female & M: Male); Episodes or recurrences: Episodes or recurrences before the use of bezlotoxumab; VAN: vancomycin; MET: metronidazole; FID: Fidaxomicin

DISCUSSION

A preliminary experience with bezlotoxumab in a population at very high risk of CDI recurrence showed high rates of cure and very good tolerance.

CDI the most frequent cause of nosocomial diarrhea and an important cause of morbi-mortality in hospitalized patients. CDI usually occurs in severely ill patients, after antibiotic prescription due to proven or suspected infections.

CDI risk of recurrence is a concerning problem. While antibiotics such as vancomycin or fidaxomicin are used to treat CDI, fecal microbiota transplant (FMT) and bezlotoxumab may have a role in preventing recurrences.

Possible benefits of the use of bezlotoxumab are that it could be available in every hospital and may be offered also to patients with contraindications for FMT or those who refuse FMT.

Bezlotoxumab has been financed in Spain for patients with high risk of recurrence.

Therefore, we selected a population with particularly high risk of r-CDI, prioritizing patients in which 3 or more risk factors were present [23].

For this purpose, we designed a score so we could choose patients for treatment with Bezlotoxumab while the hospital acquires its own experience with this monoclonal antibody.

The recurrence rates in people with risk factors for CDI reported in previous studies are up to 21-75% [24,25]. Our study, showed that in patients with 3 or more risk factors for rCDI, the rates of recurrent episodes was 21.42%, and therefore probably complies with the reduction of rCDI rates from

the clinical trial [17] (bezlotoxumab 16.5% vs. placebo 26.6%; p=0.0001). In patients with some prior recurrence, recurrence rate in the bezlotoxumab group decreased to 25%. It is important to highlight that the patients who suffer the rCDI had had 2 and 4 respectively recurrences previously, so it is probably not the best clinical scenario to use bezlotoxumab. Tolerability was excellent in all cases.

To the best of our knowledge, there are only two publications describing real-life use of bezlotoxumab, none of them from Spain. The study by Oksi et al. described their experience with 46 patients from 5 hospitals in Finland and observed a recurrence rate of CDI of 27% (78% had three or more known risk factors for recurrence of CDI) [18], and Hengel et all described 200 patients receiving bezlotoxumab,15.9% of whom experienced a rCDI [19].

Some limitations of our study need to be considered: Firstly, it was performed in only one institution with a limited sample size; and secondly, it does not have a population to compare recurrence rates.

Despite these limitations, in our experience bezlotoxumab proved to be a clinically effective drug for avoiding recurrences of CDI. The current data provide a starting point for performing future clinical trials to study the recurrence rate of CDI when using three different treatment options: 1) fidaxomicin, 2) vancomycin followed by fecal microbiota transplant (FMT), or 3) the combination of vancomycin with bezlotoxumab.

In conclusion, real-world experience on bezlotoxumab efficacy seems to be promising. All of our patients had 3 or more risk factors for CDI and 87.5% (14) of them were immunosuppressed. Bezlotoxumab was effective in the prevention of CDI recurrence in 78.57% (11/14) of the cases without side effects observed.

Bezlotoxumab could be an effective alternative for those patients who refuse FMT, those who may have contraindications to it, or in hospitals where it is not available.

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CONFLICTS OF INTEREST

Emilio Bouza, Patricia Muñoz, Maricela Valerio, Martha Kestler and María Olmedo have received fees for scientific advice or participation in scientific meetings of Astellas and MSD.

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Evaluation of a lateral flow immunoassay to detect CTX-M extended-spectrum β-lactamases (ESBL) directly from positive blood cultures for its potential use in Antimicrobial Stewardship programs

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ABSTRACT

Background. Bloodstream infections (BSI) caused by extended-spectrum beta-lactamases *Enterobacteriaceae* (ESBL-E) are associated with high rates of treatment failure and increased mortality, especially when appropriate antimicrobial therapy is delayed. Our aim was to evaluate the anticipation of ESBLs detection and the potential improvement of the time response of the Vitek 2 System (BioMérieux; France).

Methods. We compared this lateral flow immunoassay when used directly on fluid from positive blood cultures to the VITEK2 AST system. We evaluated 80 isolates, 61 tested directly on fluid from positive blood cultures and 19 tested on fluid from blood cultures spiked with known ESBL positive and negative organisms.

Results. The concordance between the CTX-LFIA and the reference method (Vitek 2) had a Cohen´s Kappa coefficient of 0.97, indicating a particularly good correlation between both compared methods.

Conclusion. This lateral flow immunoassay can be more rapid than the Vitek 2 for earlier presumptive identification of CTX- M ESBLs and can be useful to anticipate results and the adjustment of antimicrobial therapy.

Keywords: lateral flow immunoassay; extended-spectrum beta-lactamase; positive blood cultures; VITEK2

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Evaluación de una inmunocromatografía para detectar beta-lactamasas de espectro extendido CTX-M directamente de hemocultivos positivos para su potencial uso en programas de optimización de antibioterapia

RESUMEN

Antecedentes. Las bacteriemias causadas por *Enterobacteriaceae* productoras beta-lactamasas de espectro extendido (BLEE) están asociadas con altas tasas de fallo de tratamiento y mortalidad, especialmente cuando se retrasa el tratamiento apropiado. Nuestro objetivo ha sido evaluar la anticipación de la detección de estas BLEE y la potencial mejora en el tiempo de respuesta respecto al VITEK2 System (Biomerieux; Francia).

Métodos. Se comparó una inmunocromatografía para su detección con el VITEK2 AST system directamente del hemocultivo. Se evaluaton 80 aislados, 61 evaluados directamente de hemocultivos positivos y 19 de la misma manera pero inoculados con microorganismos productores y no productores de BLEE.

Resultados. La concordancia entre la inmunocromatografía y el VITEK2 AST mostró un coeficiente Kappa de 0,97, indicando una buena correlación entre ambas técnicas.

Conclusión. Esta inmunocromatografía puede ser más rápida que el VITEK2 para una identificación de BLEE tipo CTX-M y puede ser útil para anticipar resultados y ajustar la terapia antimicrobiana.

Palabras clave: inmunocromatografía; beta-lactamasas de espectro extendido, hemocultivos positivod; VITEK2

INTRODUCTION

The spread of extended-spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-E) is a growing public health threat worldwide. In Spain, since 2000, the percentage of extended-

spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* has been increasing, mostly seen in cases of urinary tract infections [1]. Bloodstream infections (BSI) caused by ESBL-E are associated with high rates of treatment failure and increased mortality, especially when appropriate antimicrobial therapy is delayed [2]. An empirically appropriate treatment is important to reduce mortality and complications [3].

To identify resistance mechanisms, such as carbapenemases and EBSLs, new molecular and non-molecular methods are being developed [4–7]. CTX-M MULTI (CTX-LFIA) (NG biotech, France) is a lateral flow immunoassay for detecting CTX- M ESBL producers. The system has been validated for use directly from colonies. Our aim was to find a diagnostic tool than can anticipate de ESBLs detection and improve the time response of the Vitek 2 System (BioMérieux; France) and if this LFIA could be implemented as microbiological tool in antimicrobial stewardship programs.

MATERIAL AND METHODS

We tested eighty isolates of *Enterobacterales* from blood cultures in this study. The isolates were the following: sixty-one consecutive routine positive blood cultures detected in our laboratory between March and June of 2019, and 19 stored gram-negative blood culture isolates, including both positive and negative ESBLs isolates, which were evaluated from spiked blood cultures. These nineteen isolates (seventeen positive ESBLs and two negative isolates) were included due to the low proportion of positive ESBLs isolates in the routine work of our laboratory. All the isolates were identified by mass spectrometry (MALDI-TOF, Bruker, Germany) following the procedures described before [8].

Susceptibility testing. Susceptibility testing was performed directly from blood cultures by determining MIC values and ESBL screening using the Vitek 2 System (BioMérieux; France) [8].

ESBL CTX-LFIA test using spiked blood cultures. For spiked blood cultures BD BACTEC TM Plus aerobic and anaerobic Culture Vials (Becton Dickinson, Madrid, Spain) were inoculated with 10 ml of blood from healthy volunteers and each bottle was inoculated with 500 µl of a suspension adjusted to 10^3 bacteria/ml in 0.9% sodium chloride and incubated at 35°C with agitation in a BACTEC FX automated blood culture system until bottles flagged positive. For control tests, the bottles were inoculated with 10 ml of blood from healthy volunteers and 100 µl of 0.9% sodium chloride.

ESBL CTX-LFIA test. The operating procedure to perform the CTX-LFIA test directly on fluid from blood cultures was the following: sample preparation followed the MALDI-TOF direct identification protocol described by Romero-Gómez et al [8]. The following procedure for inoculating the LFIA cards directly from positive blood culture bottles was done; a 4-ml aliquot was centrifuged at 140 g for 5 min. The supernatant was removed and transferred to a new tube, and then centrifuged at 16,000g for 10 min. The supernatant was discarded, and the sediment was used to make a bacterial suspension adjusted to a McFarland standard of 0.7-1. After this, one hundred microliters of the mixture were deposited in the CTX-LFIA cassette, and the result was read fifteen minutes after sample deposition as the manufacturer´s instructions describe. The test was read in the following manner: fifteen minutes after sample deposition the test line is checked versus the control line. This LFIA test has the CE marking that authorizes its marketing for in vitro diagnostics. The discordant results were corroborated by an in-house PCR to detect CTX-M.

Statistical analysis. The concordance rate between the CTX-LFIA test and our standard laboratory method was examined using the Cohen´s Kappa coefficient**.**

RESULTS

We tested eighty positive blood culture samples in the study, sixty-one samples were consecutive clinical isolates evaluated directly from positive blood cultures (Table 1), and nineteen by inoculating blood cultures with well-characterized ESBL and non-ESBL bacterial isolates (Table 1). The concordance between the CTX-LFIA and the reference method (Vitek 2) had a Cohen´s Kappa coefficient of 0.97, indicating a particularly good correlation between both compared methods. Only one blood culture (*E. coli* involved) was negative in the CTX-LFIA and positive by VITEK 2. This discrepancy was resolved by microdilution Microscan WalkAway (Beckman Coulter, Barcelona, Spain)), confirming the VITEK 2 EBSL positive result. This discordant result was corroborated by an in-house PCR that detected the CTX-M.

DISCUSSION

In this study, the performance of CTX-LFIA rapid diagnostic tests for the detection of EBSL directly on fluid from blood cultures was evaluated. These results compare favorably with the 100% of correct CTX-M identifications published recently by Bianco et al. and Bernabeu et al. [9,10]. However, we had one discordant result. We studied this isolate to ensure that it was a CTX-M isolate. The molecular analysis from colonies was positive for the CTX-M ESBL type. Therefore, this CTX-LFIA result was a false negative. We evaluated four species of *Enterobacteriaceae* that include the vast majority of our ESBL isolates (near the 90 percent of the total). CTX-M b-lactamases are predominant in Spain, and are one of the main causes of healthcare-associated ESBL-producing *E. coli* bacteremia of urinary origin in Spain [11,12]. We did not observe any additional resistance mechanisms in the routine isolates, although the CTX-LFIA can detect the CTX-M enzymes in combination with other ESBLs and other antimicrobial resistance mechanisms [9].

The performance of this CTX-LFIA directly on fluid from blood cultures offers a fast identification of ESBL-E. This tool may

Table 1 **Isolates evaluated by the CTX-LFIA test.**

Gram-negative species (N=80)	Number of isolates tested	Number of ESBL isolates tested	Number of non ESBL isolates tested	Number of ESBL isolates detected by CTX-M MULTI test	Number of negative ESBL isolates detected	Additional resistance mechanisms	Polimicrobial blood culture and additional bacterial species identified
Klebsiella pneumoniae	9	9	0	9	0	OXA 48 (1 strain)	0
Escherichia coli	9	8	1	8	1	0	0
Klebsiella aerogenes	1	0	1	0	1	0	0
Spiked blood cultures clinical isolates							
Klebsiella pneumoniae	17	5	12	5	12	0	0
Escherichia coli	42	5	37	4	37	0	2 (*P. mirabilis, K. pneumoniae*)
Klebsiella oxytoca	2	0	2	0	2	0	0
Routine prospective blood culture isolates							
TOTAL	80	27	53	26	53	0	2

be useful in elderly patients with bacteriemia/sepsis/ septic shock after a urinary tract infection to adjust the treatment as soon as the blood culture flags positive. It is described in the literature that elderly people in nursing homes had a risk around 40% higher than their community-dwelling peers of having antibiotic-resistant *Enterobacteriaceae* cultured from their urine samples [13] and almost one in five long term care facilities residents is colonized with ESBL-E [14]. Based on the results obtained in this evaluation the implementation of the CTX-LFIA in our workflow would anticipate the EBSL screening of CTX-M type at least 12 to 24 hours respect to the routine workflow implemented currently [8]. In addition, we did not observed interferences in the interpretation of the results when the CTX-LFIA is performed directly from the pellet. The additional time in the sample processing in our routine work is only the fifteen minutes of the CTX-LFIA.

As we have observed with our clinical isolates, this CTX-LFIA can be also especially useful in positive blood cultures in patients admitted in the emergency room. Reports have also described ESBL-producing *E. coli* as a cause of bloodstream infections associated with community-onset urinary tract infections [12,15]. Different prevalence of ESBL-producing bacteria has been found in many studies [16–20]. Bloodstream infections caused by ESBL-E are associated with high rates of treatment failure and increased mortality, especially when appropriate antimicrobial therapy is delayed [2]. This CTX-LFIA can be useful in sepsis/bacteriemia cases in which the patients are treated empirically with a third-generation cephalosporin. It allows escalation of the treatment 24 hours sooner than antimicrobial susceptibility testing used in our institution for positive blood cultures. This CTX-LFIA can help to ensure an appropriate antimicrobial treatment for these ESBL microorganisms sooner, which is important to reduce mortality and complications [3]. Negative results should be managed carefully, due to other ESBLs (SHV, TEM…) that can be present. In case of negative result of this LFIA, targeted or de-escalation of antimicrobial therapy must be guided by antibiogram results and not by the result of this CTX-LFIA.

We observed a great correlation between our AST system and the CTX-LFIA, although the ESBL epidemiological situation of Spain, in particular our hospital, brought on this good correlation [11]. This fact can make the CTX-LFIA an epidemiological surveillance method of ESBL in our context and can help to detect changes in the ESBL bacteriemia distribution in our hospital ecology.

In addition, this assay combined with other rapid carbapenemase detection methods might be especially useful in antimicrobial stewardship programs. The rapid identification of resistance mechanisms is one of the major efforts that the clinical microbiology laboratory should do to implement these rapid tests in the routine work of an antimicrobial stewardship program [21].

This evaluation has two limitations. Due to the low number of isolates evaluated and the low incidence of bacteriemia due to *Enterobacterales* as *Proteus* spp., *Salmonella* spp. or *Raoultella* spp., we did not evaluate isolates belonging to these species. The second limitation is the absence of other resistance mechanisms as carbapenemases or AMPc in the isolates evaluated. We only had in our evaluated isolates one additional resistance mechanism in a *K. pneumoniae* (OXA-48).

Overall, the CTX-LFIA showed good correlation with our routine instrument directly from the positive blood cultures. It can be useful to escalate treatment of bacteremia/sepsis and septic shock of community-onset correctly and promptly, although prospective studies should be performed to corroborate this issue and the utility in the real clinical setting.

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None to declare

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Brief report

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High incidence of COVID-19 at nursing homes in Madrid, Spain, despite preventive measures

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ABSTRACT

Objective. To assess the impact of COVID-19 at nine nursing homes in Madrid, Spain, during the first wave of COVID-19 infection and lockdown period when preventive measures were taken to avoid transmission among residents.

Methods. Nine hundred forty-two residents and 846 staff members from nine nursing homes participated in the study (April 18 to June 20, 2020). All participants were tested for SARS-CoV-2 in the nasopharynx by PCR and for IgG antibodies detection. Microbiological status at sampling was defined as active infection (positive PCR ± presence of antibodies), past infection (negative PCR + presence of antibodies), or naïve participants (negative PCR + absence of antibodies).

Results. Laboratory results helped classify the residents as having active infection (n=224; 23.8%), past infection (n=462; 49.1%), or being naïve (n=256; 27.1%); staff members were actively infected (n=127; 15.1%), had had a past infection (n=290; 34.2%), or were naïve (n=429; 50.7%). Overall, the percentage of participants with COVID-19 was significantly higher in residents than in staff members (72.8% *vs* 49.2%; *P*=0.001). The clinical situation of residents *vs* staff at sampling was as follows: acute manifestations compatible with COVID-19 (7.3% *vs* 3.9%; *P*<0.01) and no manifestations of infection (92.7% *vs* 96.0%; *P*<0.01). A large proportion of both asymptomatic and symptomatic residents (69.4% *vs* 86.6%; *P*=0.015) had positive PCR results (mostly alongside positive IgG determinations).

Conclusions. COVID-19 affects 75% of the residents in nursing homes in Madrid. The high impact in these settings, despite the strict restrictions adopted during the lockdown, demonstrates the ability of SARS-CoV-2 to cause outbreaks.

Keywords: COVID-19, nursing home, PCR, antibody, IgG

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Elevada incidencia de COVID-19 en residencias de ancianos en Madrid, España, a pesar de las medidas de prevención

RESUMEN

Objetivo. Evaluar el impacto de la COVID-19 en nueve residencias de ancianos en Madrid (España) durante la primera ola de infección y el período de confinamiento, cuando se tomaron medidas preventivas para evitar la transmisión en estos centros.

Métodos. Se tomaron muestras de 942 residentes y 846 miembros del personal de nueve residencias de ancianos (del 18 de abril al 20 de junio de 2020). A todos los participantes se les realizó una prueba PCR en muestra nasofaríngea, y detección de anticuerpos IgG en sangre. El estado microbiológico en el momento del muestreo se definió como infección activa (PCR positiva ± presencia de anticuerpos), infección pasada (PCR negativa + presencia de anticuerpos) o sin infección (PCR negativa + ausencia de anticuerpos).

Resultados. Los residentes tuvieron infección activa (n=224; 23,8%), infección pasada (n=462; 49,1%) o no infección (n=256; 27,1%); el personal presentó infección activa (n=127; 15,1%), infección pasada (n=290; 34,2%) o no tuvieron infección (n=429; 50,7%). En general, el porcentaje de participantes con COVID-19 fue significativamente mayor entre los residentes que entre los miembros del personal (72,8% *vs* 49,2%; *P*=0,001). La situación clínica de los residentes *vs* el personal en el momento del muestreo fue: manifestaciones agudas compatibles con COVID-19 (7,3% *vs* 3,9%; *P*<0,01) y sin manifestaciones de infección (92,7% *vs* 96,0%; *P*<0,01). Una elevada proporción de residentes tanto asintomáticos co-

mo sintomáticos (69,4% *vs* 86,6%; *P*=0,015) tuvieron resultados positivos de PCR (principalmente junto con determinaciones positivas de IgG).

Conclusiones. La COVID-19 afectó al 75% de los ancianos de las residencias de mayores de Madrid. El alto impacto en este entorno, a pesar de las estrictas restricciones adoptadas durante el confinamiento, demuestra la capacidad del SARS-CoV-2 para provocar brotes.

Palabras clave: COVID-19, residencias de ancianos, PCR, anticuerpos, IgG

INTRODUCTION

Spain has been one of the hardest hit countries by the COVID-19 pandemic [1] and the elderly population has been the most affected [2]. COVID-19 disproportionately affected residents and care workers at nursing homes during the first wave of infections that started late-February 2020 and lasted for three months [3]. The exponential increase of cases overwhelmed the Spanish healthcare system and, during the lockdown (from March 15 to June 21), family visits to nursing homes were forbidden. Despite the measures, SARS-CoV-2 kept spreading, causing multiple outbreaks with high-associated mortality rate [4].

The real impact of COVID-19 in nursing homes may be underestimated given the high number of residents or staff members who were asymptomatic and/or the lack of proper testing at the beginning of the pandemic. Epidemiological studies combining both clinical and laboratory data can help to better understand the extent and spreading of SARS-CoV-2 infections in critical settings during the pandemic. The combination of PCR testing and antibody determination helped us to describe that up to 92% and 48% of residents and staff members, respectively, were infected with COVID-19 in a nursing home in Madrid [5]. We here assessed the impact of COVID-19 at nursing homes in Madrid, Spain, during the first wave of COVID-19 infection, when first strict lockdown period and initial preventive measures were implemented.

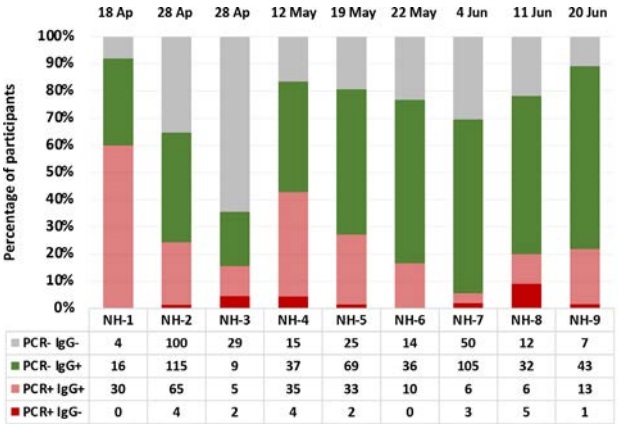
MATERIAL AND METHODS

All surviving residents and health care workers who consented to participate in the present study were included. A total of 1,788 residents and staff members from nine nursing homes located in Madrid, Spain, participated in the study between April 18 and June 20, 2020. Subjects were assessed with both a nasopharynx PCR sample for PCR testing (TaqManTM 2019-nCoV assay, Applied Biosystems, Pleasanton, CA, USA). Positive PCR results were defined as simultaneous amplification of ORF, S and N genes with Ct values lower than 37. Detection of serum IgG antibodies against the SARS-CoV-2 nucleocapsid protein was carried out in the Architect analyser using Abbott's SARS-CoV-2 IgG assay (Abbott, Abbott Park, IL, USA) following manufacturer´s instructions. The assay is based on a chemiluminescent microparticle immunoassay and determinations were considered negative or positive depending if results were < 1.4 or ≥ 1.4, respectively (cut-off index value) [6]. All samples were processed in the Microbiology and Infectious Disease Department at the Hospital General Universitario Gregorio Marañón.

Microbiological status at the time of sampling was defined as active infection (positive nasal PCR ± presence of antibodies), past infection (negative nasal PCR + presence of antibodies), or naïve participants (negative nasal PCR + absence of antibodies); participants with active or past infections were all together considered infected. Proportions were compared with Epidat v.4.2 (Consellería de Sanidade, Xunta de Galicia, Spain).

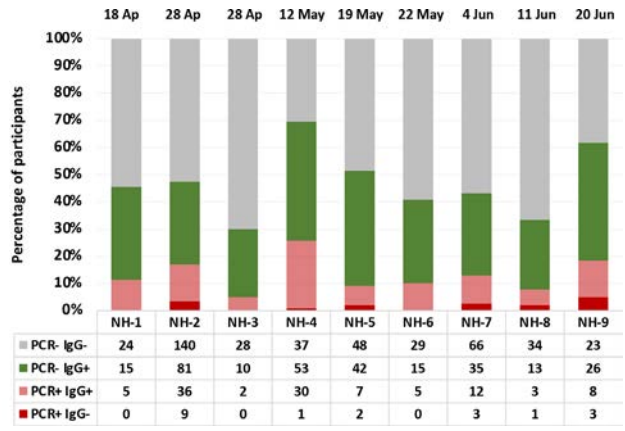
Ethics statement. This study was approved by the Ethics Committee of Hospital Gregorio Marañón (CEim; MICRO.HGUGM.2020-019).

A



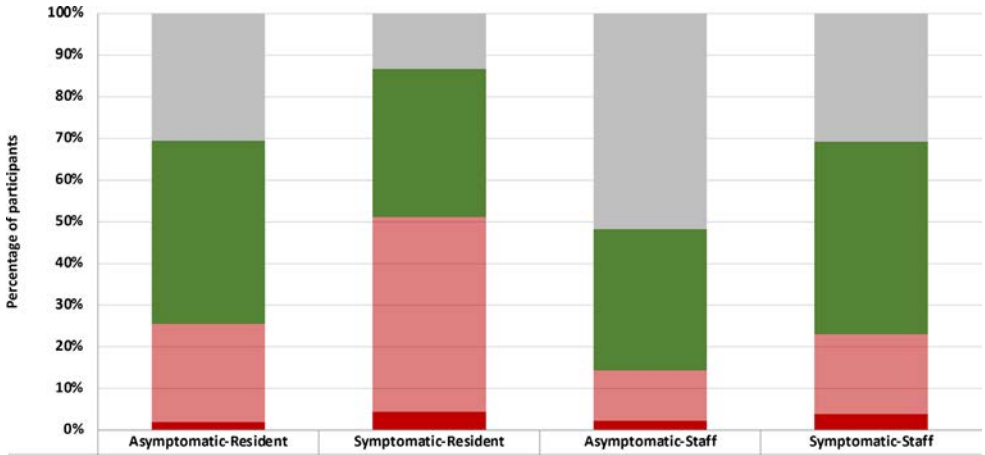
	NH-1	NH-2	NH-3	NH-4	NH-5	NH-6	NH-7	NH-8	NH-9
PCR- IgG-	4	100	29	15	25	14	50	12	7
PCR- IgG+	16	115	9	37	69	36	105	32	43
PCR+ IgG+	30	65	5	35	33	10	6	6	13
PCR+ IgG-	0	4	2	4	2	0	3	5	1

B



	NH-1	NH-2	NH-3	NH-4	NH-5	NH-6	NH-7	NH-8	NH-9
PCR- IgG-	24	140	28	37	48	29	66	34	23
PCR- IgG+	15	81	10	53	42	15	35	13	26
PCR+ IgG+	5	36	2	30	7	5	12	3	8
PCR+ IgG-	0	9	0	1	2	0	3	1	3

Figure 1 | **Percentage of residents (1A) and staff workers (1B) at each nursing home grouped as either having active infection (positive PCR ± IgGs), past and cured infection (negative PCR and positive IgGs), and naïve patients (negative PCR and negative IgGs). Dates of sample collection are also shown.**



	Asymptomatic-Resident	Symptomatic-Resident	Asymptomatic-Staff	Symptomatic-Staff
PCR- IgG-	174	6	325	8
PCR- IgG+	251	16	213	12
PCR+ IgG+	134	21	76	5
PCR+ IgG-	11	2	14	1

Figure 2 **Percentage of symptomatic and asymptomatic residents and staff workers, grouped as being actively infected (positive PCR ± IgGs), past and cured infection (negative PCR and positive IgGs), and naïve patients (negative PCR and negative IgGs) at the sampling.**

RESULTS

At sampling, 942 surviving residents and 846 staff members were assessed. Median age of the residents was 89 years (IQR: 83.7-92); 77% were female; in the case of staff workers, median age was 46 years (IQR: 38-55); 82.6% were female. The number of residents/staff workers in each nursing home was as follows: NH1 (n=50/44), NH2 (n=284/266), NH3 (n=40/40), NH4 (n=91/121), NH5 (n=129/99), NH6 (n=60/49), NH7 (n=164/116), NH8 (n=55/51), and NH9 (n=64/60). Overall, 224 (23.7%) residents and 127 (15.1%) staff members resulted positive for SARS-CoV-2 by PCR, whereas IgG determination was positive in 665 (70.6%) residents and 398 (47.0%) staff members.

Considering both laboratory results we were able to classify residents as either having active infection (n=224; 23.8%), past infection (n=462; 49.1%), or naïve (n=256; 27.1%); regarding staff members, 127 (15.1%) had active infection, 290 (34.2%) past infection, and 429 were naïve (50.7%). Overall, the percentage of infected participants was significantly higher in residents than in staff members (72.8% *vs* 49.2%; *P*=0.001). Remarkable differences in terms of infected residents among nursing homes were found (residents among 35.6% and 92.0%; staff workers among 30.0% to 69.4%; Figure 1A and 1B). As the first pandemic wave progressed, the number of affected residents increased, a pattern that was not as clear for staff workers (Figure 1).

The clinical situation at sampling for the 1,269 participants (data was unavailable in 519 participants), residents and staff workers, respectively, was as follows: acute manifestations compatible with COVID-19 (7.3% *vs* 3.9%; *P*<0.01) and no manifestations of infection (92.7% *vs* 96.0%; *P*<0.01). Notably, a large proportion of both asymptomatic and symptomatic residents (69.4% *vs* 86.6%; *P*=0.015) had PCR positive results (mostly alongside positive IgG determinations) (Figure 2). Out of the naïve resident participants (n=174), only two had had proven COVID-19 and four had had probable COVID-19 (clinically suspected but not demostrated microbiology); regarding naïve staff members (n=325) three had had proven COVID-19 and 15 had had probable COVID-19.

DISCUSSION

COVID-19 hardly hit Spain during the first wave of the pandemic starting in late February 2020. It had a profound and uneven impact in residents in nursing homes in Madrid, the epicentre of the pandemic in Spain at that time [7]. These institutions represent a setting at a high risk of COVID-19 transmission due to the advanced age of residents and their underlying conditions. SARS-CoV-2 spreading has been deeply described in nursing homes around the world, including other Spanish regions [8], other European countries [9-13], China [14] or the USA [15].

PCR testing for nursing homes residents was implemented in mid-April 2020, which makes impossible to assess COVID-19 attributable mortality before that time. Some estimates suggest that 87% of COVID-19 attributable deaths in Spain occurred among individuals aged 70 years old and above; during the first wave, 13% of all residents died from COVID-19 in Spain [16,17], such a figure rises to 22% in residents over the age of 80 years. In Madrid, 18% of nursing homes residents died from March to May 2020 [18].

Despite the strict restrictions taken during the lockdown, three quarters of the surviving residents in nursing homes in Madrid had some evidence of past or active COVID-19 disease. In contrast, the disease affected staff workers to a lesser extent. As the first wave of cases progressed, the proportion of residents affected also increased. Specific aspects of nursing homes (shared rooms or bathrooms, physically or cognitively impaired residents requiring high-demand care, rotating staff working in several facilities) may have facilitated the rapid spread of viral infections. Unfortunately, restriction policies for visitors in nursing homes implemented as part of the state of emergency declared on March 14 were insufficient to halt further transmissions [19]. The percentage of residents and staff workers who were asymptomatic at the time of sampling illustrates how insufficient the clinical presentation of the disease to control outbreaks resulted, since those asymptomatic cases could have had an important role in transmission [20].

Our study has limitations. First, not all the nursing homes were assessed at the same sampling time; second, clinical situation were not available for all participants; third, clinical situation was recorded at the sample time point and not on previous days. Finally, some nursing homes characteristics such as the proportion of care staff/residents ratio, proportion of shared rooms, among others, was not provided due to the overwhelming situation during the first wave.

In conclusion, COVID-19 affected three quarters of the surviving residents in nursing homes in Madrid, showing how devastating COVID-19 was in such facilities. The high impact suffered in these settings, despite the strict restrictions adopted during the lockdown, demonstrates the ability of SARS-CoV-2 to cause outbreaks.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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Letter to the Editor

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Neisseria meningitidis bacteraemia and SARS-CoV-2 infection: a coinfection that reminds previous epidemic outbreaks

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Sir,

We hereby report the clinical case of an 89-year-old woman who was institutionalized in a nursing home. In March 2020, she was referred to the Emergency Care due to high fever (39ºC), overall health impairment, nausea and dry cough. On physical examination, fever (38ºC) and abnormal pulmonary auscultation (right lower lung inspiratory crackles) were found. The heart rate 90 beats per minute, the blood pressure 159/70 mmHG, the respiratory rate 18 breaths per minute and the oxygen saturation 94% while the patient was breathing ambient air. There were no rashes or petechiae. No signs of neurological impairment were observed. Chest-X-ray showed right basal infiltrate consistent with pneumonia. The white-cell count was 27.940/mm^3 (89% polymorphonuclear cells), the haematocrit was 35% and the platelet count was 159.000/mm^3. A comprehensive metabolic panel was notable for a procalcitonin level of 7,47 ng/ml and the C-reactive protein level was 8,48 mg/dl. Blood cultures were collected and nasopharyngeal smear samples for seasonal flu and SARS-CoV-2 RT-PCR were negative. The patient was hospitalized and antibiotic treatment was initiated. Blood cultures yield positive for penicillin-susceptible *Neisseria meningitidis* (MIC = 0,016 mg/L) and the patient received a ceftriaxone course.

Chest CT scan was requested and confirmed lobar pneumonia in the lower right lobe (Figure 1). After 5 days of hospital stay, the fever persistence and the confirmation of a SARS-CoV-2 outbreak in the nursing home lead to a second RT-PCR, which turned out with a positive result. With a radiographic pattern suggestive of bacterial pneumonia, elevation of procalcitonin level, and in absence of neurological symptoms, the infection was interpreted as *N. meningitidis* pneumonia in the context of SARS-CoV-2 infection, although sputum samples confirming its etiology were not available. She was transferred to the respiratory isolation unit and symptomatic treatment for SARS-CoV-2 infection was initiated. No respiratory impairment was observed during hospitalization and fever disappeared after four more days. Patient was finally discharged after full recovery, normalization of analytical parameters and negative SARS-CoV-2 RT-PCR.

N. meningitidis is a Gram-negative aerobic diplococcus carried asymptomatically in up to 20% of the population [1]. Humans are the only natural reservoir of *N. meningitidis*, and the nasopharynx is the site from which they are transmitted to others by aerosol or secretions. Meningococci overcome host defenses and attach to the microvillous surface of nonciliated columnar mucosal cells of the nasopharynx, where they multiply and colonize the host [2]. Invasive disease is only developed in a minority of cases, and it represents a rare but severe event. Meningococcal meningitis is by far the most common clinical presentation, followed by bacteraemia and pneumonia. The latter was firstly described in 1918 during the wrongly called "Spanish flu pandemic" [3], during which an unusually high incidence of this complication was reported [4]. Person to person spread takes place by respiratory droplet infection. It is facilitated by some factors, like active or passive exposure to tobacco smoke, as well as by concurrent viral infections of the upper respiratory tract, because those situations enhance the formation and spread of droplets, and they also reduce the functional and mechanical integrity of the respiratory mucosa as a barrier to invasion. Viruses may also induce immune dysregulation, which increases susceptibility to bacterial infections [2]. Thus, prior studies have noted a higher incidence of meningococcal disease during viral epidemic outbreaks, especially related to *Influenza* virus [5]. These findings have been reported both at small scale (within families or classmates), but also at larger scale, identifying more virulent meningococcal infection rates and higher than usual after pandemic outbreaks (i.e. in the United Kingdom in 1957, 1976 and 1989, or in France between 1985 and 1990) [6,7]. It has been suggested that a concomitant viral infection could foster *N. meningitidis* invasion of local bloodstream caus-

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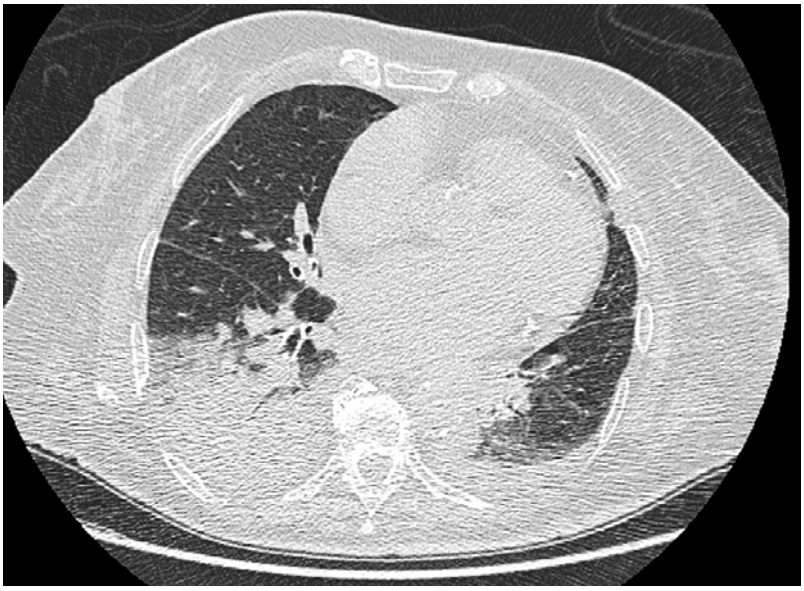

Figure 1 | **Chest CT scan showing pulmonary consolidation with air bronchogram consistent with lobar pneumonia in the lower right lobe.**

ing bacteraemia, and then dissemination of the pathogen to other tissues such as the lungs. Other possible explanations for meningococcal pneumonia include inhalation of contaminated respiratory droplets from infected patients, or microaspirations from colonized pharynx to respiratory airways [2]. In the case we present, the radiological features of the lung infiltrate were not the typical of COVID-19 pneumonia and the patient did not develop respiratory impairment, therefor it can be suspected that the pneumonia had a bacterial aetiology rather than a viral one.

In conclusion, although morbimortality of COVID-19 is closely and mainly related to inflammatory and microthrombotic lung complications, we highlight its possible association with pulmonary bacterial coinfections, both by common pathogens [8] but also by other less frequent pathogens such as *N. meningitidis*. A greater focus on the implications of this association could foster an early detection and appropriate treatment of patients who present with these complications.

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None to declare.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Letter to the Editor

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A case report. Rediscovering tuberculostatics drugs: skin rash and pyrazinamide

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Sir,

Since the use of anti-tuberculosis drugs began in 1950s, the most well-known adverse reactions to anti-tuberculosis treatment are gastrointestinal, which can lead to therapeutic non-compliance. In addition, it is known that there may be mild skin lesions, such as acne or rash, generally during the first trimester [1].

A 50-year-old male patient, native of Morocco and with habitual residence in Spain for the last 20 years, without a personal history of interest, presented with a constitutional syndrome with weight loss of 4 kilograms and intermittent fever of 2 months of evolution, accompanied by hyporexia, asthenia and predominantly nocturnal sweating. He made annual trips to Morocco and did not remember contact with animals or people sick with tuberculosis (TB).

On physical examination, he had no pathological lymphadenopathy, skin lesions, or palpable organomegaly. Serum biochemistry was normal and tumor markers were negative (CEA, CA125, Ca15-3, Ca19.9, FP, HCG). The serologies were negative for hepatotropic viruses and HIV. A thoraco-abdomen-pelvic computed tomography (CT) was performed, showing a soft tissue mass located in the posterior mediastinum at the level of D4 to D9, a 5x5x11 cm lesion, of homogeneous density, encompassing the thoracic aorta descending from the aortic arch until entering the abdominal cavity. It showed mediastinal lymphadenopathies of up to 20mm and an anterior paraspinal mass, with irregular edges that surrounded the common iliac arteries, extending through the retroperitoneum in contact with the ureters more pronounced on the right, findings suggestive of retroperitoneal fibrosis (Figure 1 A-B).

At this point, an EBUS-type bronchoscopy was requested, performing cytology aspiration punctures by esophageal routes, and biopsies by means of mediastinoscopy. Given the disparity in the results, it was decided to access by thoracostomy, which pathological result demonstrated the existence of necrotizing granulomas.

With the pathological diagnosis of presumed disseminated tuberculosis (without microbiological confirmation) empirical treatment was started with isoniazid, rifampicin, pyrazinamide and ethambutol. Ten days after the start of treatment, he had fever and bulging, confluent and elevated papule-like lesions in the thoracic, dorsal, lumbar and abdominal regions (Figure 1 C). It was interpreted as an adverse drug reaction and anti-tuberculosis drugs was withdrawn. Two days after the pharmacological withdrawal, the skin lesions disappeared, so ethambutol and isoniazid were reintroduced and after five days pyrazinamide was added to the treatment. An hour later the rash reappeared on the face region, neck and trunk.

After the suspension of all the drugs the lesions practically disappeared, having only nausea and vomiting. The analysis highlighted hyperbilirubinemia of 4.1 mg / dL at the expense of direct, GOT 2,328 IU / L, GPT 1,939 IU / L, glycemia 52 mg / dl and INR 2.02 (prothrombin time 23 sec). He scored on the Meld scale 20 points [2].

Support therapy was administered, achieving progressive clinical and analytical improvement. Due to the favorable evolution, the treatment with ethambutol was reintroduced and he was followed up in outpatient consultations.

Finally, he continued home treatment and levofloxacin was added as the second tuberculostatic drug. Currently, the patient remains stable with double antituberculous therapy with a reevaluation thoracic-abdominal-pelvic scan (CT) which shows improvement in thoracic and abdominal lesions with decreased volume and size of lymphadenopathy.

The adverse effects of treatment are varied, although the most frequent are associated with digestive intolerance, liver toxicity and others of a mild nature such as anorexia or nausea [3]. Pyrazinamide, derived from nicotinamide, was introduced

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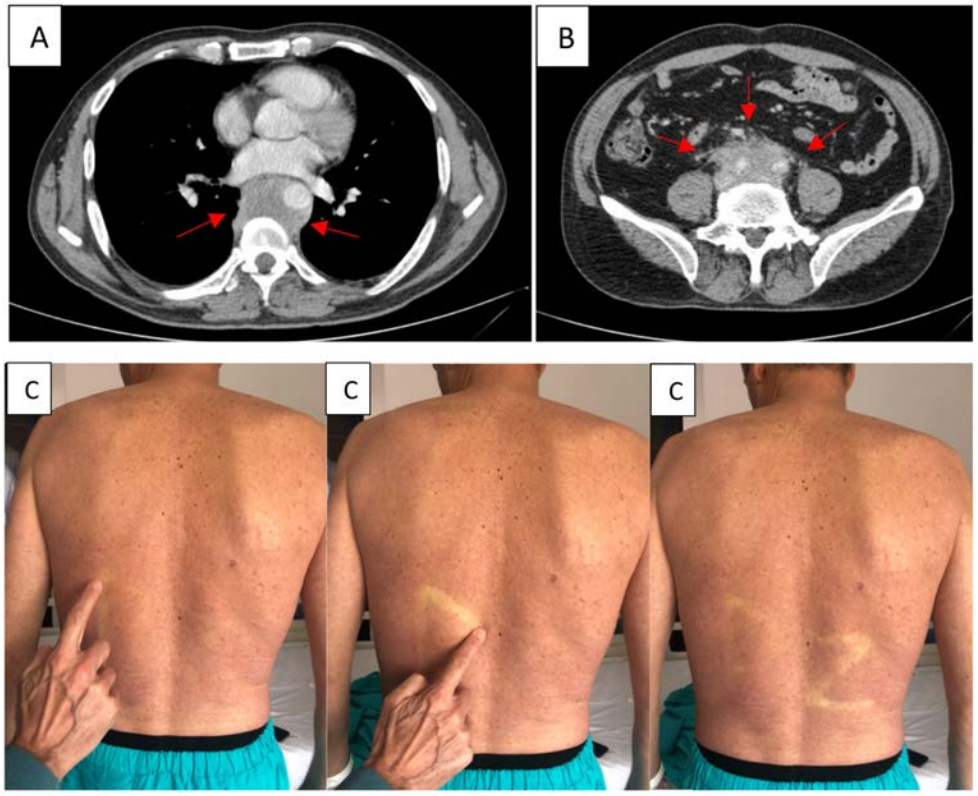

Figure 1 Thoraco CT: A soft tissue mass of thoracic location (A); Abdomen-pelvic CT: retroperitoneal fibrosis in the abdominal region (B); Extensive skin rash after taking pyrazinamide (C)

Table 1 Summary of characteristics in the bibliographic references of association between treatment with pyrazinamide and skin lesions.

Reference	Country	N	Age	Tuberculostatics	Fever	Rash	Appearance time	Withdrawal	Improvement	Reintroduction
Ribi et al [4]	Latin/ American	1	41	Isoniazid, Rifampicin, Ethambutol, Pyrazinamide	No	Yes	30 minutes	Yes	24 hours	Progressive
Perdu D et al [6]	France	1	75	Isoniazid, Rifampicin, Ethambutol, Pyrazinamide	No	Yes, erythema multiforme	26 days	Yes	5 days	Yes
Olivier C et al [8]	France	1	8	Isoniazid, Rifampicin, Pyrazinamide, Ethambutol	Yes	Yes	30 minutes	Yes	1 hour	Yes
Radal M et al [9]	France	3	64	Rifampicin, Isoniazid, Pyrazinamide	No	Yes	30 minutes	Yes	NA[a]	Yes
			65	Rifampicin, Isoniazid, Pyrazinamide	No	Yes	1 hour	Yes	Hours	Progressive
			8	Isoniazid, Rifampicin, Pyrazinamide, Ethambutol	Warm	Yes	30 minutes	Yes	1 hour	Partial

Table 1 **Summary of characteristics in the bibliographic references of association between treatment with pyrazinamide and skin lesions. (continuation)**

Reference	Sequence	Progressive doses	Rash appeareance	Features	Management	Pathologicalanatomy
Ribi et al [4]	1st Isoniazid+ rifampicin 2nd pyrazinamide	Yes.	Yes	Similar	Pyrazinamide was replaced by ciprofloxacin	No
Perdu D et al [6]	Rifampicin + pyrazinamide	No	Yes	Similar	Rifampicin was withdrawn from treatment	Yes
Olivier C et al [8]	1st Isoniazid+ rifampicin, 2nd pyrazinamide	Yes, Pyrazinamide	Yes	Similar	Lower dose treatment was maintained	No
Radal M et al [9]	1st Rifampicin, 2nd isoniazid, 3º pyrazinamide	No	Yes	Similar	Substitution of pyrazinamide for Ethambutol	No
	1st Isoniazid+ rifampicin, 2nd pyrazinamide	Yes->Pyrazinamide1/3[b] standard dose 2/3[b] standard dose y complete dose	No	NA[a]	Standard dose treatment was maintained	No
	1st Isoniazid + rifampicin, 2nd pyrazinamide	Yes->Pyrazinamide 1/3[b] standard dose y 2/3[b] standard dose	Yes	Similar	Administration at lower doses allowed maintaining the treatment	No

[a]Not applicable (no information available). [b]Reduced doses, expressed as fractions.

into the therapeutic arsenal of tuberculostatics in the 1950s. The most common adverse reaction is hepatitis, which occurs in 1-5% of cases. Skin reactions have been described with a variable proportion from 1 to 13% [4,5].

Hypersensitivity reactions can occur with any of the tuberculostatic drugs, preferably manifesting as a pruritic, macular or papular erythematous rash that predominantly affects the trunk and proximal areas of the extremities. These effects usually appear in an interval of 3-7 weeks after the start of treatment [4], being more frequent and with severity criteria in HIV positive patients [6]. Other less prevalent but more serious entities have also been described such as Steven-Johnson syndrome, toxic epidermal necrolysis, generalized pustular rash, and DRESS (Drug Rash with Eosinophilia and Systemic Symptons) syndrome [7]. However, it should be noted that the existence of a hypersensitivity reaction does not exclude that the patient may develop an anaphylactic reaction [3].

It is necessary to identify the drugs responsible for hypersensitivity reactions, for which the sequential introduction with each of the compounds at reduced doses should be carried out. In patients who initially had a severe hypersensitivity reaction, the use of much smaller doses should be prioritized [3].

There are not many reported cases of acute skin toxicity from pyrazinamide (Table 1). However, all of them show a clinical picture similar to our case, with the appearance of a sudden acute skin rash after ingestion of the drug. That is why it is important to promptly identify and withdraw the medication.

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CONFLICTS OF INTEREST

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Neumonía secundaria por *Bordetella hinzii* en paciente con infección por SARS-CoV-2

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Bordetella hinzii es un cocobacilo gramnegativo que coloniza frecuentemente las vías respiratorias de aves de corral y puede provocarles infecciones respiratorias [1]. En pacientes inmunocomprometidos, con enfermedad de base o tras exposición a aves de corral, puede comportarse como patógeno oportunista [2] y dar lugar a infecciones respiratorias, septicemia, colangitis, peritonitis, endocarditis o meningitis [3-6].

Referimos el caso de un paciente de 41 años que como único antecedente relevante presenta obesidad y dislipemia, sin tratamiento alguno actualmente.

Acude al hospital por disnea, tos y expectoración blanquecina de 24 horas de evolución. Cuatro días antes, presenta una PCR positiva para SARS-CoV-2 con mutaciones compatibles por PCR (AllplexTM, Seegene, Corea) para la variante B.1.617.2 Al ingreso se observa fiebre de 38.5ºC sin dolor torácico, vómitos, dolor abdominal ni alteración del ritmo intestinal.

La analítica sanguínea muestra: 6,800 leucocitos/µL, LDH 446 U/L en plasma, Proteína C Reactiva 19,73 mg/dL y Procalcitonina 0,14 ng/mL. La serología para microorganismos responsables de neumonías atípicas (*Legionella pneumophila, Mycoplasma pneumoniae, Chlamydophila pneumoniae* y *Coxiella burnetii*) es negativa. En la radiografía de tórax se observan infiltrados pulmonares alveolo-intersticiales bilaterales, siendo diagnosticado de insuficiencia respiratoria aguda hipoxémica secundaria a neumonía bilateral por SARS-CoV-2.

El paciente durante su estancia en Urgencias, se mantiene estable hemodinámicamente, pero con necesidad de oxigenoterapia con mascarilla reservorio, manteniendo saturación del 89% y con ligero trabajo respiratorio, por ello se decide su ingreso en la Unidad de Cuidados Intensivos donde se procede a medidas de soporte respiratorio con ventilación mecánica invasiva y soporte total debido a la severa alteración de la oxigenación que presenta. Sin embargo, no muestra signos de sobreinfección respiratoria, por tanto, no se inicia tratamiento antibiótico a su ingreso. Siguiendo nuestro protocolo hospitalario para neumonía por SARS-CoV-2, se comienza tratamiento con dexametasona, emtricitabina y tenofovir.

Tras 48-72 horas de evolución, el paciente sufre un empeoramiento clínico y radiológico, presentando broncorrea abundante, febrícula, deterioro de la oxigenación, con PAFIO2≤ 150 mmHg, leucocitosis con aumento de proteína C reactiva y un incipiente infiltrado basal derecho. Se inicia entonces tratamiento empírico con meropenem y amikacina, no asociando ningún antibiótico frente a microorganismos grampositivos debido a su baja tasa en nuestro hospital. Llaman la atención las abundantes secreciones espesas, blanquecinas y malolientes que se aspiran con dificultad y los numerosos sibilantes bilaterales. Por ello, se recogen tres muestras, dos broncoaspirados en la UMI y un fibrobroncoaspirado por parte de Neumología en días sucesivos.

En todas las muestras se observan cocobacilos gramnegativos en la tinción de Gram y se obtiene un cultivo puro de colonias transparentes y curvas en las placas de agar sangre, agar chocolate y agar McConkey. Estas se identificaron como *B. hinzii* mediante espectrometría de masas tipo MALDI-TOF (Bruker Daltonics®, Alemania) con un buen score (2.56, 2.08 y 2.06). El estudio de sensibilidad mediante epsilometría (E-test, Biomerieux®, Francia) en Mueller Hinton Medium con una suspensión del 0,5 de McFarland resulta sensible, según los puntos de corte del European Committee on Antimicrobial Susceptibility Testing (EUCAST), a piperacilina/tazobactam (CMI = 1 mg/L), imipenem (CMI = 0,75 mg/L), meropenem (CMI = 0,064 mg/L), ertapenem (CMI ≤ 0,12 mg/L) y trimetoprim/sulfametoxazol (CMI = 0,012 mg/L).

Tras el resultado de los cultivos y el antibiograma, se utilizó meropenem para tratar de forma dirigida la neumonía compli-

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cada por *B. hinzii,* presentando una evolución tórpida, pero favorable desde el punto de vista de la oxigenación, con descenso de los datos de infección y mejoría de la broncorrea de manera paulatina, cumpliendo ciclo antibiótico de 2 semanas de tratamiento y con posterior resultado negativo del broncoaspirado de control.

B. hinzii es un patógeno emergente que puede producir infecciones pulmonares. En la bibliografía, hay pocos casos descritos de neumonías por este microorganismo, la mayoría suelen estar acompañadas por otras bacterias [7,8] pero recientemente se ha publicado el primer caso de neumonía en Asia producida solo por *B. hinzii* [9]. Por ello, presentamos este caso de neumonía secundaria por *B. hinzii* en un paciente diagnosticado previamente de SARS-CoV-2, sin inmunosupresión ni exposición aviar previa y que solo presentaba obesidad y dislipemia como antecedentes. El diagnóstico microbiológico se realizó correctamente mediante el análisis proteico por MALDI-TOF y gracias al tratamiento antibiótico dirigido se consiguió la estabilización del paciente.

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Los autores declaran no tener conflicto de intereses.

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Mycoplasma y *Ureaplasma* spp. en la práctica clínica de las infecciones ano-genitales

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Hemos leído con gran interés la reciente revisión publicada por Gómez-Rufo et al [1], titulada "Implicaciones clínicas de las especies del género *Mycoplasma*" en la que se documenta de manera sistematizada el aislamiento de *Mycoplasma* y *Ureaplasma* spp. en el ser humano con el objetivo de inferir la posible patogénesis de las distintas especies. El trabajo, meticuloso y extenso, aporta información relevante en relación al potencial clínico de este tipo de aislamientos; si bien, quisiéramos hacer algunas consideraciones a este respecto.

En primer lugar, nos gustaría destacar que, pese a los matices expuestos por los autores, las técnicas de amplificación de ácidos nucleicos (TAANs) están desplazando de forma generalizada al cultivo en el diagnóstico directo y la identificación de *Mycoplasma* y *Ureaplasma* spp. en los laboratorios de microbiología clínica. Las TAANs proporcionan una mayor sensibilidad, y confieren robustez y reproducibilidad al diagnóstico de las infecciones causadas por estos microorganismos.

Por otro lado, en la presente revisión los autores describen hasta 28 especies del género *Mycoplasma* y 2 del género *Ureaplasma*, todas ellas aisladas en seres humanos y a partir de las cuales se infiere una notable relación entre estas y patología o enfermedad. No obstante, cabe puntualizar a este respecto que los hallazgos en microbiología no siempre responden a una asociación estadística, y que esta a su vez no supone una inherente relación de causalidad de la cual se deduce la implicación de la bacteria en determinadas condiciones infecciosas. En esta misma línea, nos gustaría profundizar en la evidencia relativa a la implicación de algunas de estas especies, más concretamente *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* y *Ureaplasma parvum* (antes denominadas *U. urealyticum* biovars 2 y 1, respectivamente), en síndromes ano-genitales en el ser humano. La Tabla 1 muestra un breve resumen a este respecto.

En hombres, la uretritis, tanto aguda como crónica, es la manifestación clínica más frecuentemente relacionada con la infección por *M. genitalium* [2-4]. Esta inflamación de la uretra puede cursar de forma sub-clínica o, por el contrario, desarrollar síntomas como secreción, disuria, irritación del pene o malestar uretral. Las infecciones rectales por *M. genitalium*, principalmente en hombres que tienen sexo con hombres (HSH), son frecuentes; no obstante, aunque se han descrito casos de proctitis sintomáticas relacionadas con *M. genitalium* [2,5], la mayoría cursan de forma asintomática. En este sentido, la detección de *M. genitalium* en recto debería casi exclusivamente considerarse en individuos con proctitis descartando previamente patógenos ano-rectales más frecuentes como *Neisseria gonorrhoeae*, *Chlamydia trachomatis* (especialmente aquellos genotipos de linfogranuloma venéreo LGV), *Treponema pallidum* y los Virus Herpes Simple [2]. La balanitis y la postitis son la inflamación del glande y el prepucio, respectivamente, aunque con frecuencia ocurren simultáneamente. *M. genitalium* pudiera ser causa infrecuente de balanopostitis [2,6]. Pese al carácter crónico (y a menudo subclínico) de las infecciones por *M. genitalium*, no existe una clara asociación entre la infección y la prostatitis crónica [3]. Finalmente, *M. genitalium* podría ser responsable de algunos casos de epididimitis a través de un ascenso progresivo de la bacteria a lo largo de la uretra [2,7]. La infertilidad masculina relacionada con *M. genitalium* pudiera entenderse como una complicación derivada de la epididimitis [4].

Aunque la asociación de *M. genitalium* con patología genital en la mujer es algo controvertida, la bacteria podría estar implicada en el desarrollo de determinados síndromes inflamatorios en el aparato urogenital inferior [2-4,8]. Típicamente, la secreción anormal de la vagina es producida por la vaginosis bacteriana, la vaginitis aeróbica, la candidiasis o la tricomoniasis. No obstante, otros síndromes también frecuentemente

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Tabla 1 **Asociación causal entre *Mycoplasma* y *Ureaplasma* spp. y enfermedad.**

Síndrome	*Mycoplasma genitalium*[a]	*Ureaplasma urealyticum*[b]	*Mycoplasma hominis* y *Ureaplasma parvum*
Hombres			
Uretritis	+++	++	-
Epididimitis	++	+	-
Infertilidad	+	+	+
Proctitis	++	-	-
Mujeres			
Cervicitis	++	-	-
EPI	++	+	+
Infertilidad	++	+	+

Los niveles de evidencia aplicados no se corresponden con una clasificación sistemática sino con la opinión de los autores. Niveles de evidencia: +++, alto; ++, moderado; +, bajo.
Abreviaturas: EPI, enfermedad pélvica inflamatoria. [a]La infección por *Mycoplasma genitalium* se ha asociado con algunos casos de artritis reactiva adquirida sexualmente (ARAS) o síndrome de Reiter y conjuntivitis [2]. [b]La infección por *Ureaplasma urealyticum* se ha asociado con algunos casos de ARAS [9].

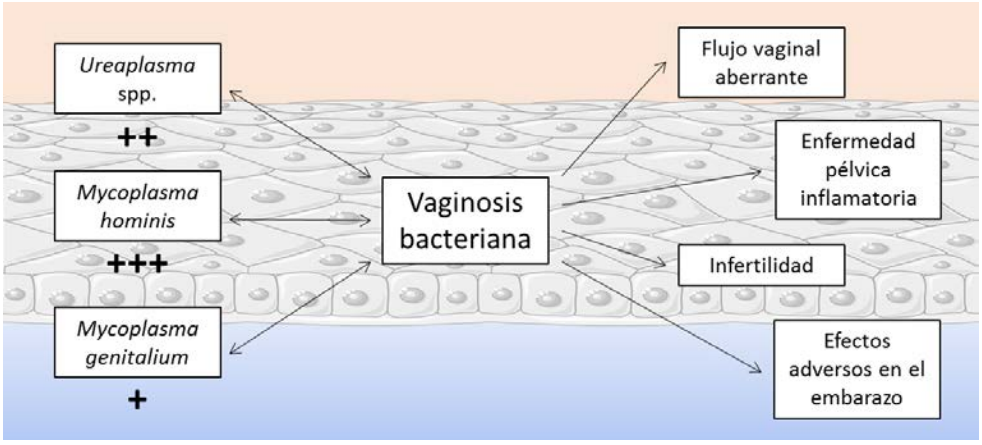

Figura 1 **Asociación entre *Mycoplasma* y *Ureaplasma* spp., vaginosis bacteriana y enfermedad en mujeres.**

La vaginosis bacteriana, como la cervicitis, se asocia con flujo y secreción vaginal, enfermedad pélvica inflamatoria, infertilidad y efectos adversos en el embarazo. En este sentido, *Ureaplasma* spp., *Mycoplasma genitalium* y especialmente *Mycoplasma hominis* pudieran estar implicados en el desarrollo de esta disbiosis y, en consecuencia, en sintomatología relacionada con la vaginosis bacteriana.
Los niveles de evidencia aplicados no se corresponden con una clasificación sistemática sino con la opinión de los autores.
Niveles de evidencia: +++, alto; ++, moderado; +, bajo.

asociados con la actividad sexual, como la cervicitis, pueden cursar de la misma manera con un flujo vaginal anómalo. A diferencia de *M. hominis*, la relación entre *M. genitalium* y las disbiosis de la microbiota vaginal es poco consistente (Figura 1) [3,8]. Por el contrario, la asociación causal entre la infección por *M. genitalium* y la cervicitis está actualmente aceptada [2-4,8]. Típicamente, la enfermedad pélvica inflamatoria (EPI) se produce por el ascenso de bacterias desde el tracto genital inferior hasta el útero, las trompas de Falopio y los ovarios. De este modo, la cervicitis (en ocasiones subclínica) es con frecuencia un antecedente necesario de esta complicación. Además de *N. gonorrhoeae*, *C. trachomatis* y microorganismos de la flora vaginal, la infección por *M. genitalium* es en este sentido reconocida como causa infrecuente de EPI [3,4,8]. Si bien

la asociación entre *M. genitalium* e infertilidad es poco consistente, esta pudiera estar relacionada con secuelas a largo plazo de infecciones persistentes/crónicas en el tracto genital superior, como la EPI [3,4,8].

A diferencia de *M. genitalium*, el potencial patogénico de *M. hominis* y *Ureaplasma* spp. en hombres y en mujeres no embarazadas está menos establecido. A día de hoy, solo las infecciones uretrales por *U. urealyticum* podrían ser consideradas relevantes en el contexto de uretritis masculinas sintomáticas, siempre y cuando otros patógenos más comunes (*N. gonorrhoeae*, *C. trachomatis*, *M. genitalium* y *Trichomonas vaginalis*) hayan sido excluidos [9].

Las posibles implicaciones de estas especies en determinados efectos adversos sobre el embarazo (y también en la infección perinatal) requieren de una discusión adicional que va más allá del propósito de esta carta.

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CONFLICTO DE INTERESES

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Sepsis por *Capnocytophaga canimorsus* en un paciente inmunocompetente

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Varón 50 años acudió a urgencias por comenzar hace 24 h con fiebre de hasta 38ºC con tiritona, artromialgias y vómitos durante la noche anterior. Como antecedentes médicos de interés destacaba un proceso previo que comenzó 30 días antes con sensación febril intermitente, odinofagia y tos, así como pérdida ponderal de unos 5 kg. Refería tener un perro cachorro como mascota que le arañaba y mordía frecuentemente, sin lesión local previa en los últimos días. Se realizó una radiografía que no mostró condensaciones, ni otras alteraciones. En analítica de sangre destacaban: proteina C reactiva de 102,22 mg/L [0,00 - 5,00 mg/L], procalcitonina de 19,08 ng/mL [0,00 – 0,5 ng/mL], leucocitosis = 33,35 x 10^3/µL [4,50 - 11,00 x 10^3] con neutrofilia = 31,88 x 10^3/µL [2,0 - 5,0 x 10^3], así como dímero D elevado de 5.230 ng/mL [0 – 500 ng/mL]. En este momento, se decidió ingreso con diagnóstico de sepsis de origen incierto para tratamiento antibiótico intravenoso con meropenem 1 g cada 8 horas por vía intravenosa. Durante el ingreso se realizó ecocardiograma transtorácico y transesofágico que no muestran valvulopatías, ni estigmas de endocarditis. El paciente mejoró progresivamente tanto clínica como analíticamente, de modo que fue dado de alta a los 7 días tras finalizar la pauta de antibiótico con meropenem sin presentar sintomatología ninguna. Se realizó un seguimiento por parte de su médico de atención primaria, que confirmó ausencia de signos y síntomas de infección durante los meses siguientes.

En urgencias se extrajo un hemocultivo, cuyos frascos anaerobios (BD BACTEC™ Lytic/10 Anaerobic/F) resultaron positivos a las 40 h. En la tinción de Gram se observaron bacilos Gram-negativos fusiformes. Se hicieron resiembras de los frascos anaerobios a agar brucella con hemina y vitamina K (Becton Dickinson, Franklin Lakes, NJ, USA), agar chocolate (Becton Dickinson, Franklin Lakes, NJ, USA) y tripticasa soya agar (TSA) con 5% de sangre de carnero (Becton Dickinson, Franklin Lakes, NJ, USA) y se incubaron en anaerobiosis y microaerofilia. Se realizó identificación directa de los frascos mediante espectrómetro de masas MALDI-TOF (desorción/ionización láser asistida por una matriz con detección de masas por tiempo de vuelo, Bruker Daltonics®) mediante el protocolo sugerido por Lagacé-Wiens PRS et al, cuyo resultado fue *Capnocytophaga canimorsus* [1]. Entre 4-6 días después, se observó crecimiento en las placas de agar chocolate (BD™, figura 1) y TSA con 5% de sangre de carnero (BD™), confirmándose la identificación de nuevo mediante espectrómetro de masas MALDI-TOF (Bruker Daltonics®). Para realizar la tipificación capsular de nuestra cepa,

Figura 1 **Crecimiento en placa de agar chocolate (Becton Dickinson, Franklin Lakes, NJ, USA) de colonias grisáceas y brillantes tras incubación durante 96h CO_2 al 5%. Se confirmó la identificación de colonias del cultivo como *C. canimorsus* mediante espectrómetro de masas MALDI-TOF.**

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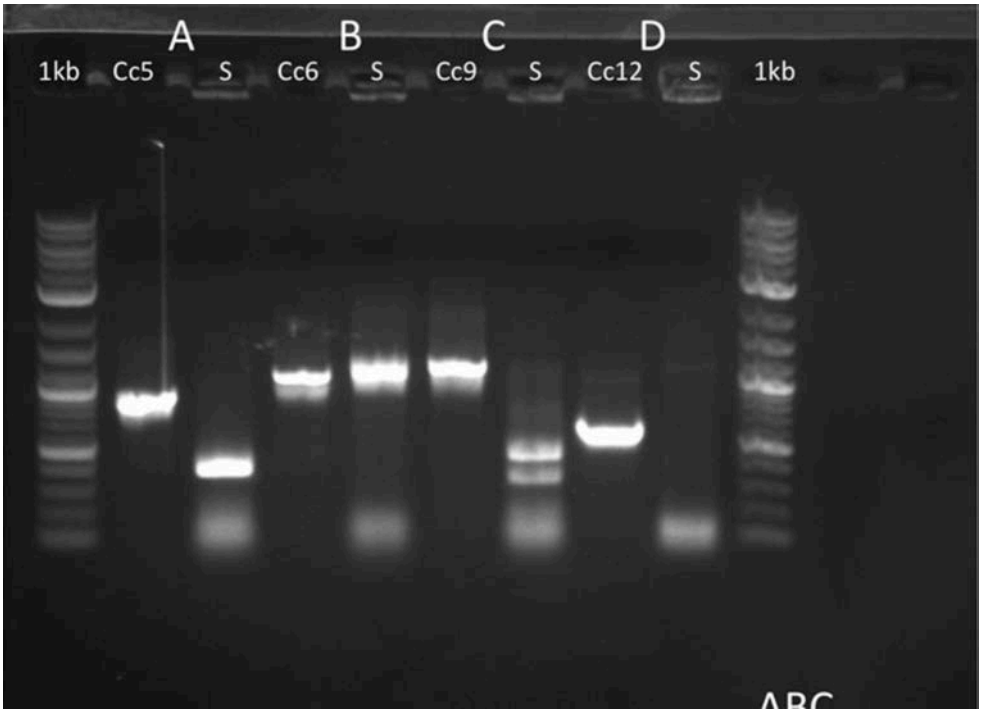

Figura 2 **Tipificación capsular por PCR de la cepa de *C. canimorsus* del caso presentado. Detección de las serovares capsulares A a D por PCR. S: cepa de *C. canimorsus* aislada en este estudio; Cc5: *C. canimorsus* cepa 5 serovar A; Cc6: *C. canimorsus* cepa 6 serovar B; Cc9: *C. canimorsus* cepa 9 serovar C; Cc12: *C. canimorsus* cepa 4 serovar D. La cepa *C. canimorsus* que aislamos en este estudio es positiva para PCR ABC (no se muestra en la figura) y B y, por lo tanto, pertenece al serovar capsular B.**

el aislado se envió a la "Unidad de Investigación en Biología de Microorganismos" de la Universidad de Namur, en Bélgica. La identificación de *C. canimorsus* se confirmó mediante secuenciación ARNr del gen 16S y la serovariedad capsular se determinó mediante el método de tipificación por PCR desarrollado por Hess E. et al. [2]. Se encontró que la cepa del caso pertenece al serovar capsular B (figura 2). La prueba de susceptibilidad antibiótica se determinó utilizando tiras de gradiente de antibióticos (o E-test^{R}) en agar Mueller-Hinton con 5% de sangre de carnero (BD^{TM}). No hay puntos de corte clínicos específicos para *C. canimorsus* en las guías de interpretación de EUCAST o CLSI. Los resultados de estas pruebas fueron los siguientes: ceftriaxona (CMI <0,064 mg/L), meropenem (CMI 0,003 mg/L), clindamicina (CMI <0,016 mg/L), amoxicilina / ácido clavulánico (CMI <0,016 mg/L) y piperacilina / tazobactam (CMI <0,016 mg/L), considerándose susceptibles en consonancia con los datos descritos en informes anteriores y siguiendo los puntos de corte de PK-PD o de bacilos gramnegativos anaerobios propuestos por EUCAST [3].

C. canimorsus es un bacilo gramnegativo anaerobio facultativo pleomórfico que crece en condiciones de anaerobiosis y microaerofilia, con un tiempo de incubación de 2 a 10 días. Habita la flora orofaríngea de perros y gatos, mayoritariamente. Habitualmente, la infección en humanos de *C. canimorsus* está precedida por lesiones producidas por perros o gatos a través de mordeduras o arañazos. Butler presenta en una revisión de 2015 datos sobre cientos de casos documentados de infecciones por este microorganismo anteriores y posteriores a 1990 en varios países [4]. En el 60% de los casos, la mordedura de un perro ocurrió antes de la enfermedad, mientras que en el 24% hubo otro tipo de contacto con perros como rasguños o contacto con su saliva. Sólo el 3% de los casos se relacionaron con lesiones producidas por gatos. En nuestro caso, el paciente había sufrido numerosos mordiscos por su mascota previamente, pero no en los días previos.

C. canimorsus se diferencia de otros bacilos gramnegativos en la composición de su membrana externa, debido a que presenta un lipooligosacárido, en lugar de un lipopolisacárido. Presenta, además, un polisacárido capsular hecho de las mismas unidades repetidas del antígeno O [5]. Se han descrito hasta la fecha al menos 7 serovares (A, B, C, D, E, L, M) en cepas aisladas de infecciones humanas, siendo los serovares A, B y C los más comunes [6]. Esto podría sugerir que estos serovares son los más virulentos para los seres humanos. El polisacárido capsular podría desempeñar un papel importante en las infecciones producidas por *C. canimorsus* probablemente en su

aparición, lo que confiere protección contra el efecto bactericida del suero, la fagocitosis y los péptidos antimicrobianos catiónicos.

Este microorganismo se puede diferenciar de otras especies de *Capnocytophaga* por pruebas bioquímicas. En nuestro caso sólo la prueba de catalasa fue positiva, aunque *C. canimorsus* suele presentar una reacción positiva de catalasa y oxidasa (como también *C. cynodegmi* y algunas cepas de *C. canis*) a diferencia del resto de especies [7]. Parece que, debido a su lento y fastidioso crecimiento, estas pruebas bioquímicas pueden dar lugar a falsos negativos. En este punto, el espectrómetro de masas MALDI-TOF, así como otras técnicas moleculares (como la secuenciación del ARNr 16S o la secuenciación completa del genoma), podrían ser útiles para acortar el tiempo de identificación de este patógeno antes de su lento crecimiento en cultivos [8].

Las infecciones se producen con más frecuencia en hombres adultos, especialmente inmunodeprimidos, asplénicos o alcohólicos [4]. En determinados casos, la infección puede derivar en complicaciones graves como coagulación intravascular diseminada, meningitis, sepsis fulminante o síndrome de Waterhouse-Friderichsen incluso en pacientes sin antecedentes médicos o inmunocompetentes, como el caso presentado [9, 10].

El tratamiento antimicrobiano de las infecciones por *C. canimorsus* consiste en antibióticos de la familia de los betalactámicos. La prueba de nitrocefina se podría utilizar para determinar cepas productoras de betalactamasas. Clindamicina es otro antibiótico distinto de los betalactámicos muy activos frente a *Capnocytophaga* spp, mientras que presentan resistencia a aminoglucósidos y polimixinas.

En conclusión, cuando un perro o gato muerde, araña o lame a una persona, especialmente población inmunodeprimida, se debe considerar *C. canimorsus* junto con otros microorganismos (que incluyen otras especies de *Capnocytophaga* como *C. cynodegmi* o *C. canis*, así como *P. multocida* y *B. henselae*) como una posible causa de infección y que ocasionalmente produzca consecuencia graves o fatales. Hay que tener en cuenta que *C. canimorsus* tiene un crecimiento más lento que otros microorganismos y requiere unas condiciones especiales de crecimiento.

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Los autores declaran la no existencia de financiación en relación con el presente artículo.

CONFLICTO DE INTERESES

Los autores declaran la no existencia de conflictos de intereses en relación con el presente artículo.

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